




Original Research Article

Synthesis, characterization and anti-inflammatory activity of an alginate-zinc oxide nanocomposite

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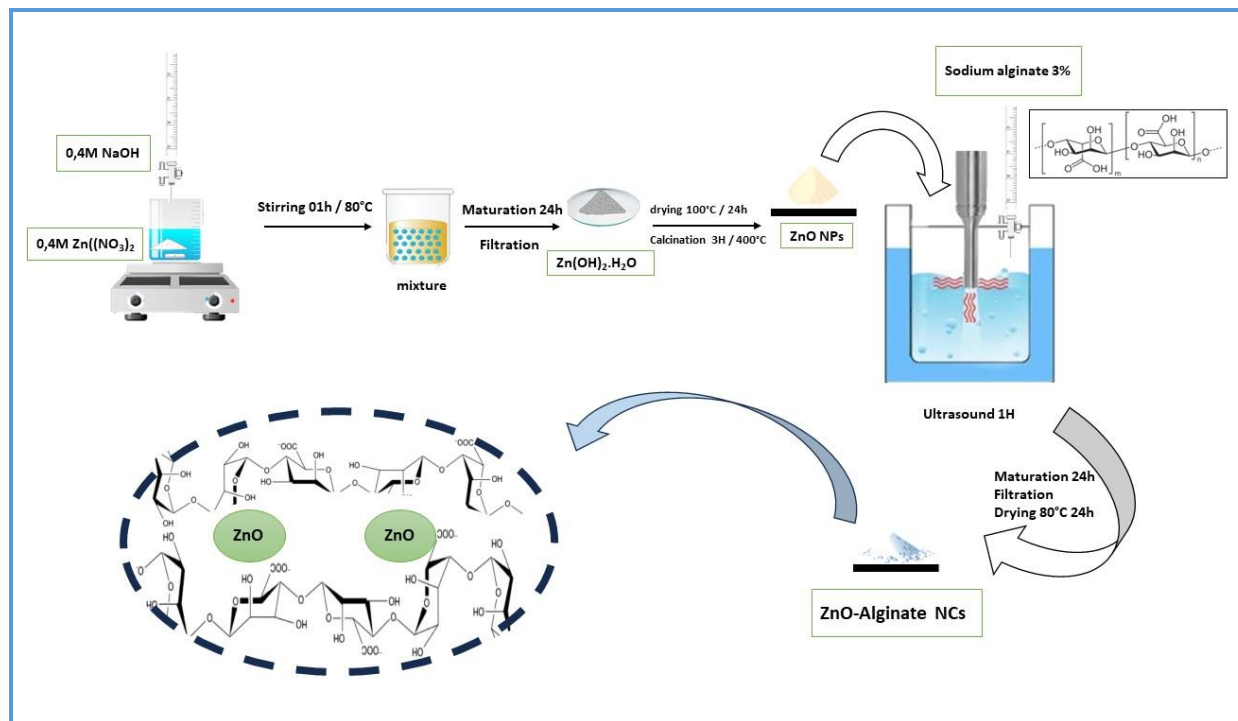
KEYWORDS

ZnO
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ABSTRACT

This research study investigated the structural features involved in synthesizing an alginate-ZnO nanocomposite with anti-inflammatory activity. Chemically synthesized ZnO nanoparticles (NPs) were combined with sodium alginate to produce a nanocomposite. The formation of the nanocomposites was confirmed through various analytical techniques, including ATR infrared spectroscopy, high-resolution field emission scanning electron microscopy, and X-ray diffraction analysis. The anti-inflammatory effect of Alg-ZnO NCs administered orally as a preventive measure was assessed by measuring the percentage of inhibition of edema induced by carrageenin in mice. Alg-ZnO demonstrated significant inhibition of carrageenan-induced paw edema.

Graphical Abstract



Introduction

Nanoparticles play a significant role in the medical and pharmaceutical industries [1]. They are extensively studied for their possible uses as specific nanocarriers and supportive tissue structures, as well as in biomaging [2]. The toxicity of zinc nanoparticles can be modulated by diverse factors, encompassing their physical and chemical properties (size, shape, stability, synthesis method, and surface chemistry) [3]. Although *in vitro* studies have shown that zinc oxide NPs can cause genetic damage and they can have the ability to cause harm to cells, there remains a dearth of information concerning their toxicity *in vivo* conditions [4]. Mice treated with ZnO nanoparticles exhibited signs of toxicity, including weight loss, passive behavior, and reduced survival [5]. Zinc, as an indispensable trace element, holds crucial significance in facilitating the proper functioning of several enzymes involved in maintaining the balance

between the interplay between oxidative and antioxidative mechanisms [6]. Zinc is increasingly recognized for its involvement in the immune system, particularly in supporting the normal development and proper functioning of cells involved in immune responses and acting as a second messenger. Recent studies have shown that inadequate level of zinc in the body leads to a systemic increase in a particular protein complex that has a significant role in the inflammatory reaction, such as NF- κ B activation. Zinc helps reduce the inflammatory state by inhibiting these protein complexes. This complex pathway is one of the key signaling pathways activated in cells during the body's reaction to inflammation [7].

Zn is a metal of block d of the periodic table of elements; this metal oxide exhibits desirable properties such as catalysis, electricity, light-induced chemical reactions and optical properties [8]. It finds applications in various

fields, including bioscience. In the scientific investigation of life and living organisms, ZnO is used as a biomimetic membrane. It can immobilize and modify proteins due to its ability to facilitate the fast exchange of electrons between atoms or molecules, such as the active sites of enzymes and the electrode surface [9]. Furthermore, ZnO offers several advantages. It exhibits significant activity in the pH neutral range (pH = 7-8) even without light. ZnO is harmless and can resist chemical changes when exposed to high temperatures and UV radiation. These properties make it a favorable material for various applications [10].

The stabilization of nanoparticles is crucial for their effective existence leading to the creation of larger and more complex compounds or assemblies [11]. Natural complex carbohydrates (polysaccharides) are widely employed for this purpose. Alginic acid is a linear copolymer featuring segments of (1→4)-linked β -D-mannuronate (M) and α -L-guluronate (G) residues, which are covalently interconnected in various sequences or blocks [12]. These monomeric units can occur in different arrangements, such as consecutive G-residues (G-blocks), consecutive M-residues (M-blocks), or alternating M and G-residues (MG-blocks). It is worth noting that α -L-guluronate is the C-5 epimer of β -D-mannuronate [13]. Sodium alginate . Sodium alginate possesses several advantageous properties, including being nontoxic, biodegradable, and biocompatible [14]. These characteristics make it highly suitable for an extensive range of uses, such as drug delivery, tissue engineering, encapsulation of compounds, and incorporation into nanocomposite structures [15]. The safety of sodium alginate, its ability to degrade naturally, and its compatibility with biological systems contribute to its suitability for these applications. The hydroxyl groups present in

the polysaccharide chains of alginate make it an attractive choice for matrix polymer synthesis [14]. Additionally, its compatibility with metal ions allows for the effective incorporation and utilization of metal nanoparticles (NPs) [16].

In preparing nanocomposites, various metal nanoparticles can be utilized, including zinc, copper, chromium and iron. These nanoparticles have the potential to be incorporated into composite materials to enhance their properties and functionality [17]. Considering all these aspects, the current study was conducted to develop a nanocomposite material using nano zinc oxide and sodium alginate, pursuing efficiency in medical applications to biomedicine. The combination of these materials holds the potential for providing enhanced anti-inflammatory properties, which this study aims to compare the influence of the administration of ZnO-alginate NCs on the anti-inflammatory activity of ibuprofen.

Experiments

Materials

Zinc nitrate ($\text{Zn}(\text{NO}_3)_2$), sodium alginate (SA), and sodium hydroxide (NaOH) were acquired from Sigma-Aldrich chemical sciences company and directly utilized as received without additional purification steps. The experiments were carried out at standard room temperature and under typical ambient reaction conditions, utilizing double-distilled water.

ZnO NP Synthesis

Zinc oxide nanoparticles (ZnO NPs) were prepared using the precipitation method. A 0.4M solution of NaOH and $\text{Zn}(\text{NO}_3)_2$, each with a volume of 200 mL, was prepared and stirred for 60 min under heating at 80 °C to achieve a

homogeneous mixture. The $\text{Zn}(\text{NO}_3)_2$ solution was then subjected to ultrasound treatment while the NaOH solution was added slowly until a white precipitate was observed. The container was covered with parafilm tape and left to mature for 24 h, forming two phases. The

precipitate was filtered through centrifugation and rinsed thrice with deionized water and ethanol to remove impurities. The resulting sediment was dried for 24 h at 100 °C and calcined in an electric furnace for 3 h at 400 °C to obtain white ZnO powder (Figure 1 and 3).

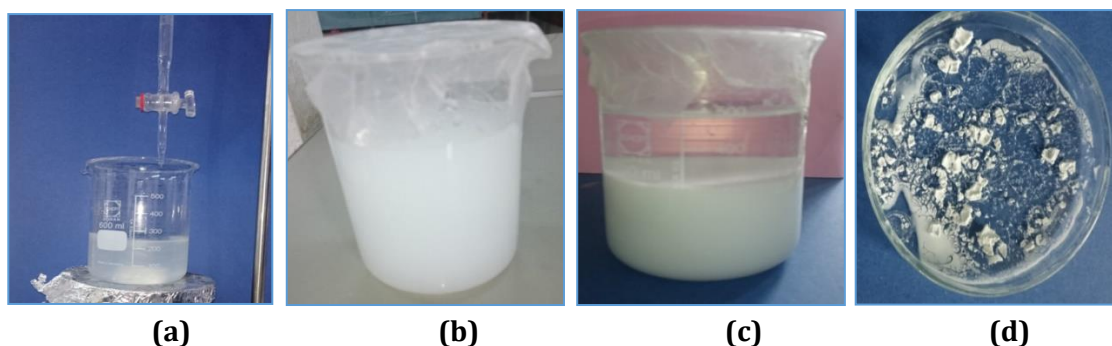


Figure 1. The main steps of synthesis of ZnO NPs, (a): mixture under ultrasound, (b): before maturation, (c): after maturation, (d): dried sample

Alginate-ZnO Nanocomposite Synthesis

ZnO-Alginate nanocomposites (NCs) were synthesized by creating a mixture under ultrasound waves. Two solutions, one containing ZnO nanoparticles (10 mg/mL) and the other with 3% alginate, were prepared in a beaker of 200 mL and placed under stirring for 60 min to ensure a homogeneous mixture (2g of ZnO and 6g of SA). The ultrasound probe was

immersed in the container with the ZnO NCs solution, and the alginate solution was added dropwise until a white frost-like precipitate formed. Subsequently, the container was sealed with parafilm tape and allowed to mature for 24 h, forming two phases. The precipitate was filtered using centrifugation and washed, and the obtained sediment was subsequently dried for 24 h at 80 °C, yielding white ZnO-Alginate powder (Figure 2 and 3).



Figure 2. The final white powder of ZnO-Alginate NCs

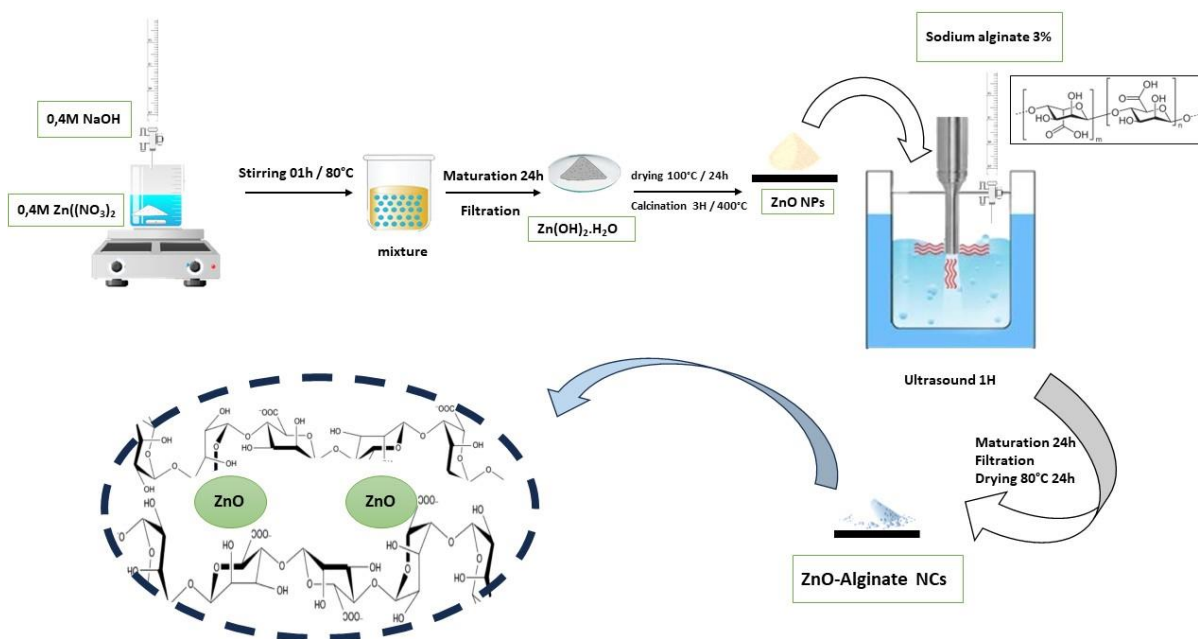


Figure 3. Preparation of the ZnO-Alginate nanocomposites

Characteristics

The characteristics of the alginate-ZnO nanocomposite were studied by attenuated total reflection (ATR) spectroscopy in the range 450–4000 cm^{-1} (Perkin Elmer), Scanning electron microscope SEM, and X-ray Diffraction (XRD).

Anti-inflammatory test

Animals

The experimental studies were conducted using male Swiss albino mice weighing between 25 and 30g. The mice were obtained from the animal facility at the Pharmacy Department of the Faculty of Medicine in Oran. Before the experiments, the animals underwent an adaptation and preparation period where they had unrestricted access to standard food and water. They were also acclimatized to a 12-hour light/dark cycle and a favorable ambient

temperature of 25 °C for 15 days before being utilized in the different experiments.

Drugs

Ibuprofen (MERINAL®) and ZnO- Alginate NCs were suspended *ex tempore* in deionized water.

Acute toxicity test

To evaluate our material's acute toxicity, four groups, each consisting of five mice, were utilized. The mice in the different groups received a single oral dose of the following prepared suspensions: 0.5 mL of NaCl 0.9% (control group), 0.5 mL of Ibuprofen (82 mg/Kg), 0.5 mL of Ibuprofen (82 mg/Kg) combined with ZnO-Alginate (3, 075 mg /kg), and 0.5 mL of ZnO-Alginate (3, 075 mg /kg). The mice were closely observed for signs of intoxication and mortality rates at 5, 30, and 45 minutes, as well as at 1, 2, 4, and 6 h after the

suspension administration. Subsequently, the mice were monitored once daily for seven days.

Determination of the anti-inflammatory activity

In the design of the experiment, the various groups received different suspensions orally (po) 30 minutes before the injection of carrageenan (as shown in Table 1). The control group was administered only the vehicle with an equal volume of 0.5 mL 0.9% NaCl. The test groups received the prepared suspension containing ZnO-Alginate, or ZnO-Alginate in combination with Ibuprofen. The reference

group, on the other hand, received the Ibuprofen suspension. Before the experiment, the mice underwent a 24-hour fasting period, with free access to drinking water.

In order to induce paw oedema in mice, a carrageenan solution was injected subplantarily (intra-articularly) into the right hind paw. This injection was performed one hour after the oral administration of the suspension (Figure 4 and 5). The procedure resulted in inflammation, which was subsequently observed to be reduced in the presence of the substance possessing anti-inflammatory activity [18].

Table 1. Steps of the anti-inflammatory activity

Groups T	Control Groupe	Reference group	Test group 1 (Ibuprofen + ZnO- Alginate)	Test group 2 (ZnO-Alginate)
T ₀ : Administration per Os	0.5 mL 0.9% NaCl	0.5 mL Ibuprofen (82 mg/Kg)	0.5 mL : Ibuprofen (82 mg/Kg) + ZnO- Alginate (3,075 mg/kg)	0.5 mL ZnO- Alginate (3.075 mg/kg)
T ₃₀ : subplantar injection	0.025 mL carrageenan solution (1%)	0.025 mL carrageenan solution (1%)	0.025 mL carrageenan solution (1%)	0.025 mL carrageenan solution (1%)



(a)



(b)

Figure 4. The anti-inflammatory activity, (a): oedema induction, (b): oedema measurement

Oedema measurement

The development of oedema was measured using the digital vernier caliper (1300E.PB

FACOM). The measurements and recording of paw diameters were conducted after the first, second, third and fourth hour from the

carrageenan injection. The formula used to calculate the percentage of oedema inhibition was as follows:

$$\text{Oedema inhibition \%} = (N - N_x * 100) / N$$

where N is the paw diameters measured 1, 2, and 3 h after injection of carrageenan to the control group (paw diameters at the beginning) and N' is the paw diameters measured 1, 2, and

3 h after injection of carrageenan to the test groups (paw diameters at the beginning).

Statistical analysis

In the carrageenan-induced hind paw oedema test, the obtained data were evaluated using the two-way analysis of variance (two-way ANOVA).

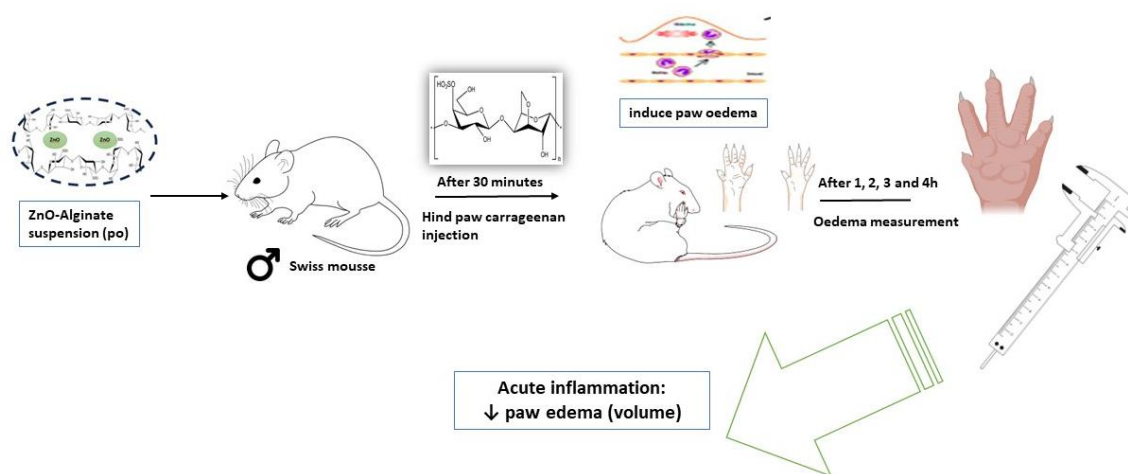
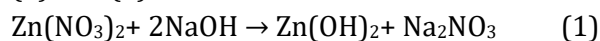


Figure 5. Potential anti-inflammatory effect of ZnO-Alginate nanocomposites in carrageenin-induced paw oedema

Result and Discussion

Fabrication of ZnO-Alginate nanocomposite signifies one of the best nanomaterials in the pharmaceuticals field for biomedical applications. During the typical process, ZnO-Alginate was synthesized by precipitation method where ZnO NPs were stabilized over sodium alginate. The possible NPs ZnO was synthesized utilizing zinc nitrate and sodium hydroxide. The possible reactions are shown in Equations (1) and (2). The development of ZnO-Alginate was schematically shown in Figure 3. The possible reactions are shown in Equations (1) and (2).



ATR-IR Analysis

The ATR-IR spectra of the ZnO NPs, sodium alginate, and ZnO-alginate nanocomposite were obtained in the range of 450–4000 cm^{-1} (Figure 6). The ATR-IR spectra of sodium alginate (Figure 6) revealed a broad peak at 3228 cm^{-1} corresponding to its hydrogen-bonded OH group. Asymmetric and symmetric stretching of the $-\text{COO}$ alginate group was observed at 1598 and 1407 cm^{-1} , respectively [19]. In addition, sodium alginate showed two characteristic peaks at 1044 and 1025 cm^{-1} corresponding to the C-O stretching vibration of its polysaccharide structure. The peak at 750 cm^{-1} corresponds to Na-O bond vibration [20]. In the ZnO NP spectrum, The peak at 1607 cm^{-1} and

3478 cm^{-1} corresponded to the flexural vibrations of the H_2O molecules. The bands at 459 and 717cm^{-1} suggested the tension mode between metal and oxygen [21,22].

The spectrum of the ZnO-Alginate nanocomposite exhibited peaks that corresponded to a combination of the characteristic bands of alginate biopolymer and ZnO nanoparticles (Figure 6). This observation indicated the presence of strong interactions

between the alginate biopolymer and ZnO nanoparticles, suggesting the formation of an optimal nanocomposite structure. An additional peak distinctive for Zn–O–Zn vibrations was noticed at 619 cm^{-1} with a slight shift in the frequency of peaks corresponding to ZnO and sodium alginate. This observation suggests that nano ZnO forms a composite bond with sodium alginate, indicating their interconnected bonding nature [10].

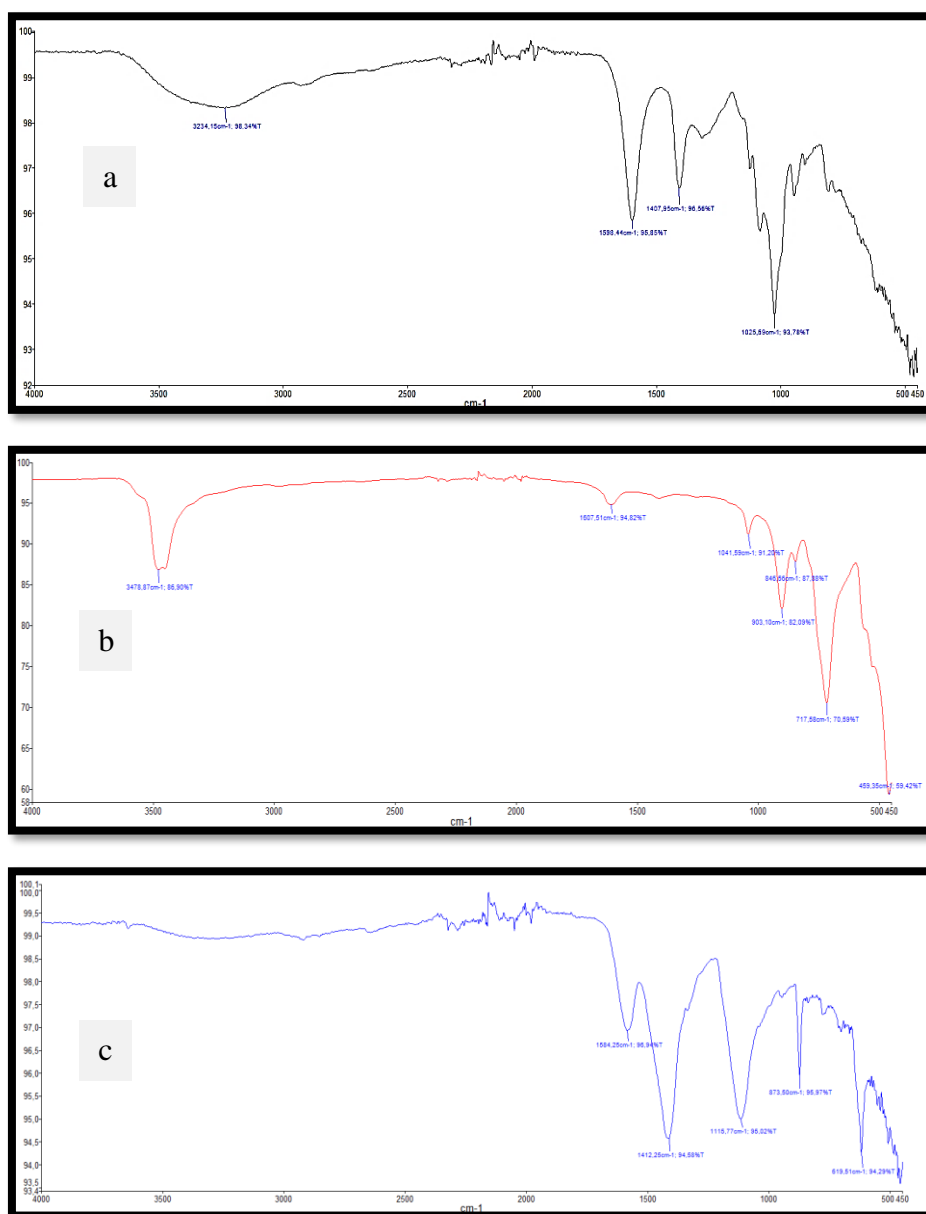


Figure 6. ATR-IR spectra of (a): sodium alginate, (b): ZnO NPs, and (c): ZnO-alginate nanocomposite

SEM Analysis

SEM (Scanning Electron Microscopy) helped provide detailed information on the morphology of the synthesized nanocomposites ZnO-Alginate. The SEM image of the synthesized nano alginate-ZnO was presented in Figure 7. The image exhibited the rod-like structure of zinc oxide nanoparticles, demonstrating excellent alignment.

The SEM images of the ZnO-alginate nanocomposites at magnifications of 3 μm , 5 μm and 10 μm are given in Figure 7. The morphology of the NCs studied with high-resolution SEM. The SEM images provided clear

evidence of the presence of ZnO nanoparticles on the surface of the alginate, illustrating the distribution of nano ZnO over the polymer .

Granulometry analysis

The distribution of particles based on their size in the ZnO-Alginate nanocomposite is depicted in (Figure 08) obtained through laser granulometry. We have noticed that the cumulative undersize distribution Q3(x) (50%) with particles volume smaller than 56 nm; and the cumulative undersize distribution Q3(x) (100%) with particles volume smaller than 214 nm (Figure 8).

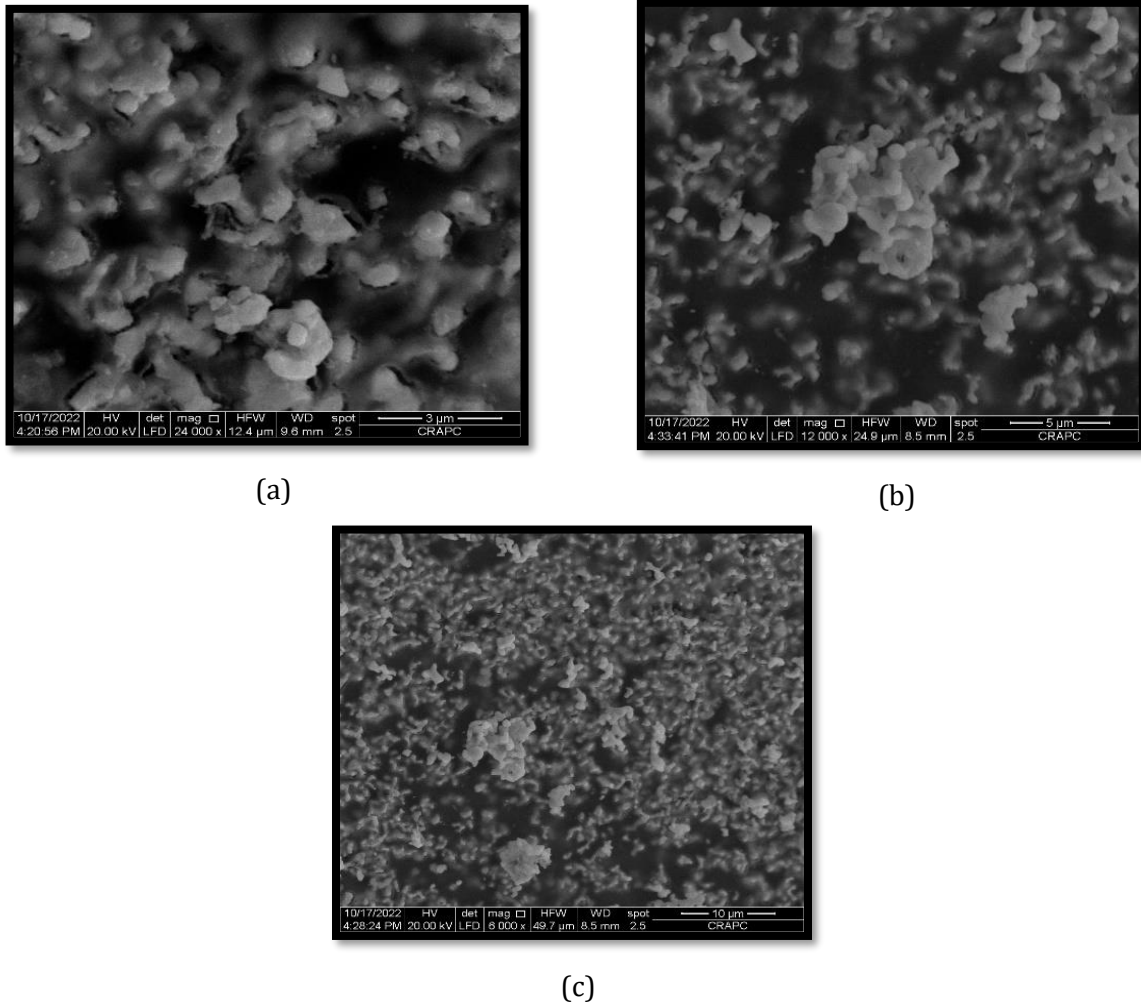


Figure 7. SEM micrographs of the ZnO-alginate nanocomposite at various magnifications (a: 3 μm , b: 5 μm , c: 10 μm)

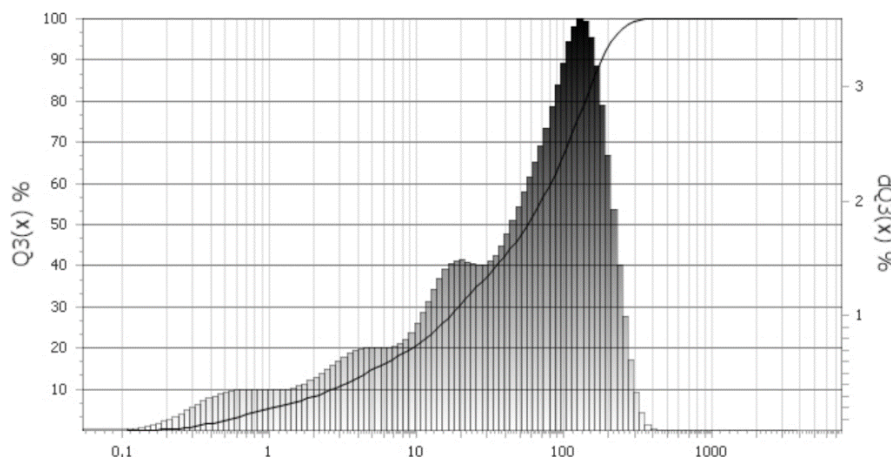


Figure 8. The size distribution of alginate–ZnO nanocomposite

XRD Analysis

The X-ray diffraction study verified that the synthesized material was indeed ZnO, and the diffraction peaks aligned with the standard JCPDS (card no. 36-1451) data, validating the

crystalline nature of the sample. The pattern of pure zinc oxide displays diffraction peaks corresponding to the principal crystalline planes of zinc oxide, indicating its well-defined crystal structure: (1 0 0), (0 0 2), (1 0 1), (1 1 0), (1 0 3), (2 0 0), (0 0 4) and (2 0 2) (Figure 9)

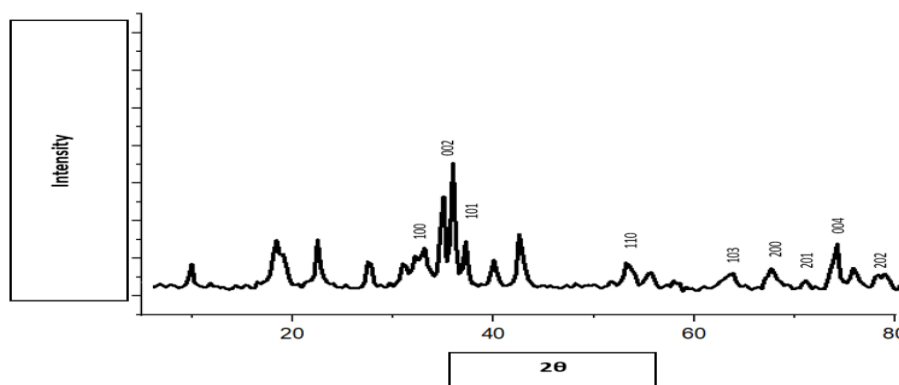


Figure 9. X-Ray diffraction spectra of alginate–ZnO nanocomposite

Anti-inflammatory activity

Acute toxicity test

The oral administration of the different solutions (0.5 mL of NaCl 0.9% (control group), 0.5 mL of Ibuprofen (82 mg/Kg), 0.5 mL of Ibuprofen (82 mg/Kg) combined with ZnO-Alginate (3, 75 mg/kg), and 0.5 mL of ZnO-Alginate (3, 75 mg/kg)) to mice did not induce

any signs of acute toxicity during the 06-hour observation and the 7 days (Table 2).

Determination of the anti-inflammatory activity

Ibuprofen, ZnO-Alginate NCs and the association (Ibuprofen + ZnO-Alginate) inhibit the carrageenan-induced paw oedema at the second, third and fourth hour after the administration of carrageenan (Figure 10). The

inhibition was significant only after the third hour for the reference group (Ibuprofen) and the group test 1 (association) by 37,91% and 39,45%, respectively, and for the three groups after the fourth hour by 52,10% (very significant) for the reference group, 33,23% (significant) for the test group 2 and 64,29% (very significant) for the test group 1 (Figure 10).

Zinc exhibits anti-inflammatory properties, reducing inflammation, and antioxidative effects, combating oxidative stress. Its anti-inflammatory properties are primarily

attributed to its involvement in inhibiting the NF-κB pathway [23]. By inhibiting this pathway, the production of pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, cyclooxygenase (COX), and nitric oxide synthase is reduced [24]. Moreover, the association (Ibuprofen + Alg-ZnO) shows significant inhibition for the carrageenan-induced paw oedema. The data from the current study and previous research suggest that the anti-inflammatory activity of zinc is influenced by its chemical compound and the specific form in which it is utilized [25,26].

Table 2. Toxicity test of different solutions over 6 h and during 7 days

	Control Groupe	Reference group	Test group 1 (Ibuprofen + ZnO-Alginate)	Test group 2 (ZnO-Alginate)
Increase of the activity	(-)	(-)	(-)	(-)
Convulsion	(-)	(-)	(-)	(-)
Coma	(-)	(-)	(-)	(-)
Death	(-)	(-)	(-)	(-)

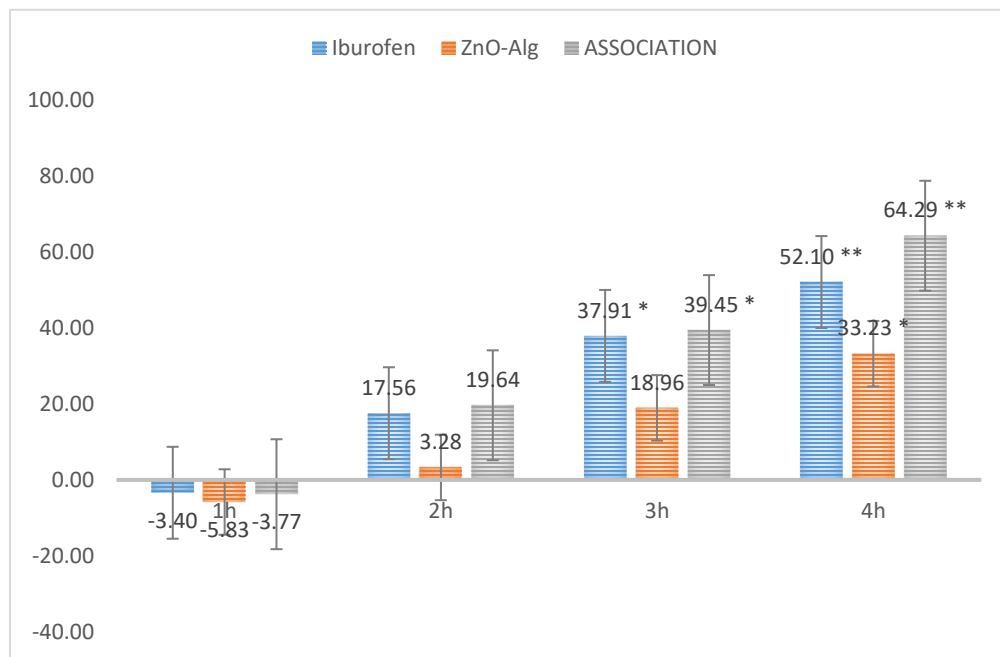


Figure 10. Influence of alginate-ZnO nanocomposite on the anti-inflammatory effect in the carrageenan-induced hind paw oedema test. The results are expressed as a percent (%) of oedema inhibition. * p<0,05; ** p<0,01; indicate the results statistically significant

Conclusion

The ZnO nanoparticles (NPs) synthesized using the precipitation method and the sodium alginate exhibited optimal purities as being used as a precursor. By properly combining the sodium alginate and ZnO NPs, ZnO-Alginate nanocomposites were produced. These interactions were confirmed through ATR-IR spectroscopy, SEM, granulometry and XRD analysis. The successfully fabricated ZnO-Alginate nanocomposites showed significant anti-inflammatory activity in inhibiting oedema. The biological effects may be beneficial, but the nanoparticles may cause oxidative stress in cells and organs. Introducing nanoparticles through administration can potentially trigger immunological and inflammatory responses in organisms.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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