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What is "Open Peer Review" process?

An "Open Peer Review" process is making the details of all review process (including reviewers, associate editors, and EICs comments) as "Public" as it is agreed by EIC, Authors, and reviewers.

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- Authors' satisfactions from the review process: Increases honesty between authors and reviewers
- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

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Peer Review Report for "Preparation and Physicochemical Characterization of Ofloxacin-Loaded Alginate-Chitosan Nanogel: Comparison to Ofloxacin-Loaded Alginate Nanogel"

Author(s): Masoume Mohammadi GhanatGhestani, Bahareh Sabeti, Saeed Manoochehri

Review Timeline:

Submit Date: 8 Oct 2025

Revised Date: 15 Dec 2025

Accepted Date: 7 Jan 2026

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFeree : Reviewer

12 Oct 2025

1. Inconsistency in Particle Size Data:

- a. Abstract (Page 3): Reports sizes as "150 nm and around 300 nm" for Alg-NG and Alg-Chi NG, respectively.
- b. Results (Page 10): Reports "70 nm" for Alg-NG and "150 nm" for Alg-Chi NG.
- c. Discussion (Page 11): Refers to a "mean hydrodynamic diameter of 70 nm" for Alg-NG.
- d. Figure 1: The graphs are labeled for "alginate nanogel (a)" and "alginate-chitosan nanogel (b)" but the caption and text do not clarify which size distribution (intensity, volume, number) is presented. The values in the graphs (D50 ~70nm for Alg-NG and

~150nm for Alg-Chi NG) seem to match the Results section, contradicting the Abstract.

These items indicated that, the authors must carefully revise the entire manuscript to ensure all particle size data (in the abstract, results, and discussion) are consistent and accurately reported. The caption for Figure 1 must specify the type of distribution shown.

2. Lack of Statistical Analysis in this manuscript:

- The manuscript states that experiments were done in triplicate (e.g., for EE and LC). However, no standard deviations (SD) or standard errors (SE) are reported for any quantitative data (particle size, PDI, zeta potential, EE, LC, swelling, drug release). All data must be presented as mean $\pm$ SD. Statistical analysis should be performed to confirm the significance of the differences observed between the Alg-NG and Alg-Chi NG formulations.

3. Zeta Potential Interpretation:

- The zeta potential of Alg-Chi NG is reported as -16 mV, which is a relatively low value and suggests colloidal instability. The authors mention the nanoparticles had (strong stability) but no data (like stability over time) is provided to support this claim.

4. Swelling Study:

The result that the plain Alg-NG "disappeared quickly" in phosphate buffer (pH 7.4) is a critical finding but is not discussed in depth. This indicates very rapid dissolution/disintegration, which has major implications for its drug release profile and suitability as a sustained-release carrier. The discussion should be expanded to explain why the Alg-NG disintegrates (weak ionic network in neutral pH) and how the chitosan coating prevents this.

5 · The abstract mentions "Levofloxacin" as a keyword, but the study uses Ofloxacin. This is a critical error and must be corrected.

6 · The introduction is comprehensive but could be more concise. The specific hypothesis and novelty of using the internal gelation method for this particular alginate-chitosan system could be stated more explicitly.

7 · Section 2.1.1: The sentence "Alginate nanogels (Alg-NGs) were prepared by emulsification/internal gelation (17)." is immediately followed by "while the Alg-NG was formed through a chelate reaction...". This is repetitive. Rephrase for clarity · Section 2.1.2: The phrase "without proceeding" is unclear. Please rephrase this sentence for better understanding.

8 · Table 1: The table is mentioned in the text but is not included in the provided pages. Please ensure it is present.

9 · Figure 5: The Y-axis label is missing. It should be "% Cumulative Drug Released" or similar. Please revise this figure.

10 · The quality and resolution of all figures (1-5) should be checked to ensure they are publication ready. Please revise this graph in final version.

11 · The manuscript requires English and grammar editing. There are numerous grammatical errors in this manuscript.

Dear editor

The article exhibits several grammatical and technical issues that hinder its clarity and effectiveness. Firstly, there are notable grammatical errors, including poor sentence structure and inconsistent verb tenses, which disrupt the flow and coherence of the text. Additionally, punctuation mistakes further complicate the reader's understanding. On the technical side, the article frequently employs specialized terminology without proper definitions, potentially alienating readers unfamiliar with the subject matter. Furthermore, the presentation of data through graphs and tables lacks clear labeling, making it difficult to interpret the findings accurately. Addressing these issues is essential for enhancing the overall quality and professionalism of the article.

1.gramatical issues:

Sentences often start with "Therefore". Try alternatives like "Thus or Hence".

The abbreviations FT, FESEMTh, COOH, CaCO, IKA, CO, IR, FESEM, API, FTIR, TPP, MO, III, USA, CaCl, II, FTIRFTIR, NH, and UV occur only a few times each. Since abbreviations are hard to decrypt, just spell them out each time. It is easier to read a few words than to search for meanings of abbreviations.

You used some difficult words like "elucidate", "purchase". Try using simple synonyms, like "explain", "buy" because most readers of scientific papers are not native English speakers.

Line 6. Replace "utilize" with simple "use".

Line 7. Consider rewriting the sentence with "has been shown" in active voice, e.g. "we showed that".

Line 12. Consider shortening "in order to" as just "to".

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Line 37. In American English, word "anatomical" is spelled as "anatomic".

Line 42. Try to rewrite without "...and so on". It might be too informal and vague if other items in the list are unclear. Either list all important items or generalize the remaining, for example "Voyager flew past Mars, Jupiter, and other planets.

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Line 62. Replace "facilitate" with simple "help". According to The Craft Of Scientific Writing: "Words such as facilitate are pretentious".

Line 67. The word "highly" rarely highly contributes to better understanding. Consider removing it or, if important quantifying it.

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Line 67. Avoid constructions with "it is" since they obscure the main subject and action of a sentence.

Line 160. Consider rewriting the sentence with "was shown" in active voice, e.g. "we showed that".

Line 172. Percent sign "%" should follow numerals without a space, i.e. 1%

Line 177 and 181. Most journals prefer shortening the word "Figure" as "Fig." if it is not opening the sentence.

2. Technical issues

Clarity of Material Specifications: Ensure that all materials are clearly described, including their sources and any relevant specifications (e.g., purity, grade). For example, when mentioning sodium alginate, clarify the significance of the M/G ratio and inherent viscosity.

Consistency in Terminology: Maintain consistency when referring to materials and methods. For instance, use either "chitosan" or "1% W/V chitosan" throughout to avoid confusion.

Emulsification Process: Provide more detail on the emulsification process, such as the duration and speed of stirring. Clarify why a specific speed (400 rpm) was chosen and whether this affects the properties of the nanogel.

Acidic Medium Preparation: Explain the rationale for using glacial acetic acid and how it contributes to the gelation process. It may also be useful to specify the final pH achieved and its importance for the reaction.

Chitosan Addition: Describe the method of incorporating chitosan into the alginate nanogel more thoroughly. Explain the significance of the 3:1 ratio and how it was determined.

Dissolution and pH Adjustment: Provide details on the pH adjustment process. Why is a pH of 4.8 chosen? Discuss its relevance to the interaction between chitosan and alginate.

Homogenization Process: Specify the duration of homogenization and the rationale behind this step. Discuss how this affects the final properties of the nanogels.

Measurement Techniques: Clarify the settings used for the Dynamic Light Scattering (DLS) measurements, including temperature and sample preparation specifics.

Zeta Potential Measurement: Explain how the zeta potential measurements correlate with the stability of the nanogels. Discuss the significance of the observed zeta potential values in relation to the expected behavior of the formulations.

FTIR Analysis Details: Provide more context regarding the FTIR analysis. For instance, explain how the spectra were interpreted and what specific peaks indicate about the interactions between alginate and chitosan.

Sample Preparation for FESEM: Detail the process used to prepare samples for FESEM analysis. Mention any specific conditions (e.g., drying methods) that could affect the morphology of the nanogels.

Calculation Methods: Clearly outline the methods used to calculate EE and LC.

Consider providing a brief rationale for why these metrics are important for evaluating nanogel performance.

Statistical Analysis: Mention any statistical methods used to analyze the data obtained from the EE and LC measurements. Specify if results were expressed as mean $\pm$ standard deviation.

-Swelling Measurement Method: Describe the gravimetric method in more detail. Explain how the weights were measured and the significance of the swelling ratio in the context of drug delivery.

Release Study Setup: Provide more detail about the setup for the in vitro release study. Explain the choice of dialysis membrane, and how its molecular weight cutoff impacts the study.

Kinetic Model Evaluation: Clarify how the different kinetic models were evaluated. Consider including a brief explanation of each model and why they are relevant for interpreting drug release kinetics.

Reproducibility: Ensure that all methods are described in sufficient detail to allow for reproducibility. This includes specifying equipment used, conditions maintained, and any calibration procedures followed.

Statistical Rigor: Include information on the sample size and any statistical tests used to analyze the data. This is crucial for validating the significance of your findings.

Formatting and Clarity: Use consistent formatting for headings and subheadings. Ensure that all sections are clearly labeled and that the methodology flows logically from one step to the next.

Keywords: The keywords "levofloxacin" are written instead of "ofloxacin", which should be corrected. The keyword "eye infection" should be removed because you did not conduct in vivo studies and it is not appropriate as a keyword in your study.

Particle size: Why is the particle size of ofloxacin-loaded leg-NG listed as 70 nm in line 155, but it is 150 nm in the abstract?

Limitation: This study lacks any cellular or in vivo studies, and this is recognized as a limitation of this study, so it should be mentioned as a limitation in the text.

REFEREE : Associate Editor

19 Oct 2025

Dear editor in-chief

According to the reviewers' feedback, the manuscript contains several grammatical and technical errors that must be addressed. These issues hinder the clarity and professionalism of the writing, necessitating a comprehensive revision. It is essential

to correct grammatical mistakes, refine technical content, and ensure the overall structure is coherent and logical. By implementing these changes, the manuscript will not only meet the reviewers' expectations but also enhance its quality and likelihood of acceptance for publication.

REFeree : EIC

20 Oct 2025

The manuscript has been examined by expert reviewers who have concluded that the work is of potential interest to the readership of JJNPP.

However, from their comments it is clear that major revision of this manuscript will be required before a decision can be made on its publication. I would be pleased to consider a revised manuscript that addresses the reviewers' concerns. Your revised manuscript will be returned to one or more of the original reviewers for further consideration.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Reviewer 0:**1. Inconsistency in Particle Size Data:**

a. Abstract (Page 3): Reports sizes as "150 nm and around 300 nm" for Alg-NG and Alg-Chi NG, respectively.

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These items indicated that, the authors must carefully revise the entire manuscript to ensure all particle size data (in the abstract, results, and discussion) are consistent and accurately reported. The caption for Figure 1 must specify the type of distribution shown.

The particle size was corrected and highlighted in the revised manuscript. The particle size of ofloxacin-loaded alginate nanogel was approximately 70 nm, while the particle size of ofloxacin-loaded alginate-chitosan nanogel was around 150 nm. The caption of Fig. 1 was also revised and highlighted.

2. Lack of Statistical Analysis in this manuscript:

• The manuscript states that experiments were done in triplicate (e.g., for EE and LC). However, no standard deviations (SD) or standard errors (SE) are reported for any quantitative data (particle size, PDI, zeta potential, EE, LC, swelling, drug release). All data must be presented as mean $\pm$ SD. Statistical analysis should be performed to confirm the significance of the differences observed between the Alg-NG and Alg-Chi NG formulations.

It was corrected and highlighted in the revised manuscript.

3. Zeta Potential Interpretation:

• The zeta potential of Alg-Chi NG is reported as -16 mV, which is a relatively low value and suggests colloidal instability. The authors mention the nanoparticles had (strong stability) but no data (like stability over time) is provided to support this claim.

As added and highlighted in the manuscript, the zeta potential values of the alginate-chitosan nanogel are -16 ± 0.9 mV, which differs from the outer surface in comparison to the alginate nanogel (-150 ± 6.2 mV). This difference could indicate that chitosan is incorporated into the alginate nanogels through the surface by an oily medium containing Span80, resulting in the zeta potential of -16 ± 0.9 mV.

Stable nanoparticles usually exhibit a zeta potential greater than 30 mV, as observed in alginate nanogels. However, when the zeta potential of alginate-chitosan nanogels decreased to -16 ± 0.9 mV, their stability was compromised, resulting in slight particle aggregation. This finding is further corroborated by the particle size measurements of the two types of nanogels. Although the nanobeads had **relative** stability, the negative charge of nanoparticles could impress the amount of exposure to the cell surface (22).

4. Swelling Study:

The result that the plain Alg-NG "disappeared quickly" in phosphate buffer (pH 7.4) is a critical finding but is not discussed in depth. This indicates very rapid dissolution/disintegration, which has major implications for its drug release profile and suitability as a sustained-release carrier. The discussion should be expanded to explain why the Alg-NG disintegrates (weak ionic network in neutral pH) and how the chitosan coating prevents this.

The manuscript has been revised and highlighted to clarify that a determined amount **of freshly prepared** alg- nanogels and alg-chit nanogels was weighed and subsequently soaked in a phosphate buffer at a pH of 7.4 for the swelling test. Additionally, freeze-dried nanogels were used in the drug release study.

It appears that the presence of water may reduce the mechanical properties of both types of nanogels, which can explain the differences observed between the swelling and the drug release studies.

As added and highlighted in the revised manuscript, "The deprotonation of alginate functional groups of freshly prepared alginate nanogels at pH 7 leads to a high anionic charge density. This charge exhibits electrostatic repulsive forces, which increase and expand the volume of the nanogels. As a result, rapid swelling occurs, and alginate nanogels disappear (1). Using **freshly prepared nanogels** can accelerate penetration of fluid to the polymeric gel, leading to quick relaxation of the macromolecular chain (2). To modify the swelling behavior, chitosan is decorated onto the alginate nanogel, creating freshly prepared alginate-chitosan nanogels. The interaction between the functional groups of chitosan and alginate delays the swelling process, resulting in only 80% swelling after 5 min soaking in phosphate buffer pH 7. "

(1): Suhail M, Fang C-W, Chiu I-H, Khan A, Wu Y-C, Lin I-L, et al. Synthesis and evaluation of alginate-based nanogels as sustained drug carriers for caffeine. ACS omega. 2023;8(26):23991-4002.

(2): El-Feky GS, El-Banna ST, El-Bahy G, Abdelrazek E, Kamal M. Alginate coated chitosan nanogel for the controlled topical delivery of silver sulfadiazine. Carbohydrate polymers. 2017;177:194-202.

5 · The abstract mentions "Levofloxacin" as a keyword, but the study uses Ofloxacin. This is a critical error and must be corrected.

When selecting "ofloxacin" in the MESH structure, it was automatically replaced with "levofloxacin." This correction was highlighted in the manuscript, but it cannot be confirmed on the site.

6 · The introduction is comprehensive but could be more concise. The specific hypothesis and novelty of using the internal gelation method for this particular alginate-chitosan system could be stated more explicitly.

It was revised and highlighted in the manuscript.

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It was corrected and highlighted in the revised manuscript.

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Reviewer 1

Dear editor

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Line 177 and 181. Most journals prefer shortening the word "Figure" as "Fig." if it is not opening the sentence.

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2. Technical issues

Clarity of Material Specifications: Ensure that all materials are clearly described, including their sources and any relevant specifications (e.g., purity, grade). For example, when mentioning sodium alginate, clarify the significance of the M/G ratio and inherent viscosity.

It was corrected and highlighted in the revised manuscript.

Consistency in Terminology: Maintain consistency when referring to materials and methods. For instance, use either “chitosan” or “1% W/V chitosan” throughout to avoid confusion.

It was corrected and highlighted in the revised manuscript.

Emulsification Process: Provide more detail on the emulsification process, such as the duration and speed of stirring. Clarify why a specific speed (400 rpm) was chosen and whether this affects the properties of the nanogel.

As added and highlighted in the manuscript, according to preliminary study and the following study (3), 400 rpm speed was chosen which makes uniform flow in the vessel, in addition to making narrower size distribution.

(3) Silva CM, Ribeiro AJ, Figueiredo M, Ferreira D, Veiga F. Microencapsulation of hemoglobin in chitosan-coated alginate microspheres prepared by emulsification/internal gelation. *The AAPS Journal*. 2005;7:E903-E13.

Acidic Medium Preparation: Explain the rationale for using glacial acetic acid and how it contributes to the gelation process. It may also be useful to specify the final pH achieved and its importance for the reaction.

The interaction between alginate and calcium occurred in the alginate nanogel through emulsification/internal. After preparing the emulsion, acidic paraffin containing glacial acetic acid was added to the emulsion. Glacial acetic acid has a low water content, so by adding acidic paraffin to the emulsion, the ratio of water to oil in emulsion remained unchanged. Additionally, the acidic medium is suitable for the dissociation and dissolution of calcium, which is necessary for the interaction between calcium and alginate.

Also, regarding to specifying the final pH, it is more accurately stated and highlighted in this way in the revised format: “after 15 min emulsification of the sample, the pH of the emulsion was adjusted to 4.7-5 by slowly adding acidic paraffin to the W/O emulsion. An acidic medium is necessary for the dissociation and dissolution of calcium. After 40 min, the alginate nanogel was prepared. In the following, nanogels were centrifuged, washed to remove excess oil, and purified.”

Chitosan Addition: Describe the method of incorporating chitosan into the alginate nanogel more thoroughly. Explain the significance of the 3:1 ratio and how it was determined.

As added and highlighted in the manuscript, several methods were used to incorporate chitosan into the alginate nanogel, including the reaction of alginate and chitosan with or without tripolyphosphate in different mass ratios (3), which resulted in microparticles. However, despite experimenting with various proportions of chitosan to paraffin and different concentrations of alginate to chitosan, the Fourier transform infrared spectroscopy results did not indicate the formation of a polyelectrolyte complex between alginate and chitosan, which might be due to the difference in molecular weight of chitosan or the method of preparation (4). Hence, based on preliminary studies, the proportion of chitosan to alginate was chosen as 3:1 for further experiments.

(3) Silva CM, Ribeiro AJ, Figueiredo M, Ferreira D, Veiga F. Microencapsulation of hemoglobin in chitosan-coated alginate microspheres prepared by emulsification/internal gelation. *The AAPS Journal*. 2005;7:E903-E13

(4) Kyzioł A, Mazgała A, Michna J, Regiel-Futyra A, Sebastian V. Preparation and characterization of alginate/chitosan formulations for ciprofloxacin-controlled delivery. *Journal of Biomaterials Applications*. 2017;32(2):162-74.

Dissolution and pH Adjustment: Provide details on the pH adjustment process. Why is a pH of 4.8 chosen? Discuss its relevance to the interaction between chitosan and alginate.

According to Kyzioł et al. study (4) as added and highlighted in the manuscript, stated that the optimum pH for incorporating alginate with chitosan by polyelectrolyte complex is approximately 4.5, which should be maintained throughout the reaction process. This is because the pKa of sodium alginate is around 4, while the pKa of chitosan is around 6.5. Hence, a pH of 4.8 was chosen for the incorporation of alginate nanogel with chitosan, as also mentioned in the alginate-chitosan synthesis.

(4) Kyzioł A, Mazgała A, Michna J, Regiel-Futyra A, Sebastian V. Preparation and characterization of alginate/chitosan formulations for ciprofloxacin-controlled delivery. *Journal of Biomaterials Applications*. 2017;32(2):162-74.

Homogenization Process: Specify the duration of homogenization and the rationale behind this step. Discuss how this affects the final properties of the nanogels.

As added and highlighted in the manuscript, for preparation of paraffinized chitosan, aqueous chitosan was emulsified in the oily medium. Span80 was used as an emulsifier. Hence, 24.5 mL of prepared chitosan was emulsified in the equivalent volume of paraffin containing 0.4% W/V Span80, and stirred for 10 min to make a paraffinized chitosan.

Measurement Techniques: Clarify the settings used for the Dynamic Light Scattering (DLS) measurements, including temperature and sample preparation specifics.

As added and highlighted in the manuscript, this device features a small immersion probe. The sample was dispersed in 5 mL of deionized water at 25 °C, allowing the probe to access the sample. The light emitted by a laser beam was guided to the optical probe by a fiber; this is focused into the sample through the protective sapphire window. The laser light reflected by the protective window and the 180° backscattered light were collected and guided through a fiber to a detector by a “Y” optical splitter.

Zeta Potential Measurement: Explain how the zeta potential measurements correlate with the stability of the nanogels. Discuss the significance of the observed zeta potential values in relation to the expected behavior of the formulations.

The zeta potential values of the alginate-chitosan nanogel are $-16 \text{ mV} \pm 0.9$, which differs from the outer surface in comparison to the alginate nanogel. This difference could indicate that chitosan is incorporated into the alginate nanogels through the surface by an oily medium containing Span80, resulting in the zeta potential of $-16 \text{ mV} \pm 0.9$.

Stable nanoparticles usually exhibit a zeta potential greater than 30 mV, as observed in alginate nanogels. However, when the zeta potential of alginate-chitosan nanogels decreased to -16 mV , their stability was compromised, resulting in slight particle aggregation. This finding is further corroborated by the particle size measurements of the two types of nanogels.

FTIR Analysis Details: Provide more context regarding the FTIR analysis. For instance, explain how the spectra were interpreted and what specific peaks indicate about the interactions between alginate and chitosan.

As added and highlighted in the revised manuscript, the Fourier transform infrared spectroscopy (FT-IR) spectra of sodium alginate, sodium alginate nanogel, chitosan and alginate/chitosan composites are presented in Figure 3. The FT-IR spectrum of sodium alginate shows a characteristic band near 1024 cm^{-1} , which is attributed to C–O–C stretching vibrations of its

saccharide framework. Moreover, the bands at 1606 cm^{-1} and 1409 cm^{-1} are assigned to the asymmetric and symmetric stretching vibrations of carboxylate ($-\text{COO}^-$) salt groups, respectively. The chitosan spectrum displays a broad and intense band at 3360 cm^{-1} , which can be attributed to the combined effects of O–H stretching, N–H stretching, and intermolecular hydrogen bonding within the polysaccharide structure. A strong absorption band observed at 2870 cm^{-1} corresponds to C–H stretching vibrations of the chitosan backbone. Additionally, two characteristic peaks at 1645 cm^{-1} and 1593 cm^{-1} are assigned to C=O stretching of the amide I (NHCO) group and N–H bending vibrations of the NH_2 group, respectively. Bands appearing at 1422 cm^{-1} and 1374 cm^{-1} are attributed to N–H stretching of the amide and ether linkages and to the amide III band, respectively. Other distinctive peaks of chitosan include those at 1151 cm^{-1} , corresponding to the (1–4) glycosidic bond, 1026 cm^{-1} related to C–O–C stretching within the glucose ring, and 1064 cm^{-1} associated with CH–OH vibrations in cyclic structures.

In the chitosan/alginate spectrum, a broad absorption band appears at 3375 cm^{-1} , indicating interactions arising from the combination of chitosan and alginate. These spectral modifications suggest that the negatively charged carboxylate groups of alginate interact electrostatically with the positively charged ammonium groups of chitosan, leading to the formation of a polyelectrolyte complex. Further evidence of ionotropic gelation is provided by the shift of the chitosan amide I peak from 1645 cm^{-1} to 1602 cm^{-1} . Additionally, the asymmetric and symmetric stretching bands of alginate $-\text{COO}^-$ groups shift to 1536 cm^{-1} and 1457 cm^{-1} , respectively, confirming complex formation.

Sample Preparation for FESEM: Detail the process used to prepare samples for FESEM analysis. Mention any specific conditions (e.g., drying methods) that could affect the morphology of the nanogels.

It was corrected and highlighted in the revise manuscript.

Calculation Methods: Clearly outline the methods used to calculate EE and LC. Consider providing a brief rationale for why these metrics are important for evaluating nanogel performance.

The methods were corrected and highlighted in the manuscript.

As added and highlighted in the manuscript, during the alginate-calcium reaction in alginate nanogel preparation, a loose network may cause leakage of ofloxacin, a hydrophilic molecule, through pores. Hence, using chitosan could reduce the permeability of the nanogel and improve its mechanical properties (4).

(4) Kyzioł A, Mazgala A, Michna J, Regiel-Futyra A, Sebastian V. Preparation and characterization of alginate/chitosan formulations for ciprofloxacin-controlled delivery. *Journal of Biomaterials Applications*. 2017;32(2):162-74.

Statistical Analysis: Mention any statistical methods used to analyze the data obtained from the EE and LC measurements. Specify if results were expressed as mean $\pm$ standard deviation.

There were corrected and highlighted in the manuscript.

Swelling Measurement Method: Describe the gravimetric method in more detail. Explain how the weights were measured and the significance of the swelling ratio in the context of drug delivery.

The manuscript has been revised to clarify that a determined amount of **freshly prepared alg-nanogels and alg-chit nanogels** was weighed and subsequently soaked into a phosphate buffer (pH: 7.4) for the swelling test. Additionally, freeze-dried nanogels were used in the drug release study. It appears that the presence of water may reduce the mechanical properties of both types of nanogels, which can explain the differences observed between the swelling and the drug release studies.

As added and highlighted in the revised manuscript, “The deprotonation of alginate functional groups of freshly prepared alginate nanogels at pH 7 leads to a high anionic charge density. This charge exhibits electrostatic repulsive forces, which increase and expand the volume of the nanogels. As a result, rapid swelling occurs, and alginate nanogels disappear (1). Using **freshly prepared nanogels** can accelerate penetration of fluid to the polymeric gel, leading to quick relaxation of the macromolecular chain (2). To modify the swelling behavior, chitosan is decorated onto the alginate nanogel, creating freshly prepared alginate-chitosan nanogels. The interaction between the functional groups of chitosan and alginate delays the swelling process, resulting in only 80% swelling after 5 min soaking in phosphate buffer pH 7. “

Release Study Setup: Provide more detail about the setup for the in vitro release study. Explain the choice of dialysis membrane, and how its molecular weight cutoff impacts the study.

The Molecular weight of alginate used in the preparation of alginate nanogel ranged from 12 to 14 kDa, and the molecular weight of chitosan varied between 50 to 190 kDa. Hence, a dialysis membrane with a cutoff of 12 kDa was selected. This choice ensured that chitosan could not pass through the membrane, while the release of ofloxacin from the nanogel could pass through easily.

The molecular weights of alginate and chitosan were mentioned in the material section of manuscript.

Kinetic Model Evaluation: Clarify how the different kinetic models were evaluated. Consider including a brief explanation of each model and why they are relevant for interpreting drug release kinetics.

As added and highlighted in the manuscript, the release kinetics of the drug molecule from the polymeric nanogels were studied. Various kinetic models were employed to analyze drug release from two types of nanogels, including zero-order, first-order, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell models to assess the release mechanism. The selection of the most suitable kinetic model was based on the adjusted R^2 value being close to 1.

Reproducibility: Ensure that all methods are described in sufficient detail to allow for reproducibility. This includes specifying equipment used, conditions maintained, and any calibration procedures followed.

The items were checked, and all necessary materials were added to the manuscript.

Statistical Rigor: Include information on the sample size and any statistical tests used to analyze the data. This is crucial for validating the significance of your findings.

It was added and highlighted in the revised manuscript.

Formatting and Clarity: Use consistent formatting for headings and subheadings. Ensure that all sections are clearly labeled and that the methodology flows logically from one step to the next.

It was added and highlighted in the revised manuscript.

Keywords: The keywords "levofloxacin" are written instead of "ofloxacin", which should be corrected. The keyword "eye infection" should be removed because you did not conduct in vivo studies and it is not appropriate as a keyword in your study.

When selecting "ofloxacin" in the MESH structure, it was automatically replaced with "levofloxacin." This correction was highlighted in the manuscript, but it cannot be confirmed on the site.

Particle size: Why is the particle size of ofloxacin-loaded leg-NG listed as 70 nm in line 155, but it is 150 nm in the abstract?

The particle size was corrected and highlighted in the revised manuscript. The particle size of ofloxacin-loaded alginate nanogel was approximately 70 nm, while the particle size of ofloxacin-loaded alginate-chitosan nanogel was around 150 nm. The caption of Fig. 1 was also revised and highlighted.

Limitation: This study lacks any cellular or in vivo studies, and this is recognized as a limitation of this study, so it should be mentioned as a limitation in the text.

It was added and highlighted in the revised manuscript.

OPEN PEER REVIEW

Revision (1)

REFEREE : Reviewer

22 Dec 2025

The authors provided appropriate responses to the reviewer's questions, and corrections were made.

REFEREE : Associate Editor

7 Jan 2026

The Ms is now suitable for publication in JJNPP

REFEREE : EIC

7 Jan 2026

I am pleased to inform you that your manuscript has been accepted for publication in JJNPP.

Your work underwent a rigorous peer-review process, and the reviewers and editorial board were impressed by the originality, scientific rigor, and relevance of your findings. We believe your contribution will be of significant interest to our readership and will advance the field of natural pharmaceutical products.

Before publication, our production team will contact you regarding final formatting, proofs, and any necessary administrative steps. Please ensure that all authors have approved the final version of the manuscript and that any required ethical or copyright documentation is in order.

On behalf of the editorial board, I congratulate you and your co-authors on this excellent work and thank you for choosing to publish in JJNPP.