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OPEN PEER REVIEW

A Novel Platform for Controlled Drug Delivery and Dye Removal; Green Synthesis and Characterization of Honey-Incorporated Cu/Al Layered Double Hydroxide (LDH) Nanocomposite

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About this report

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1 Original submission

1 comment · 6 Nov 2024

Mahmoud Gharbavi Reviewer

MINOR REVISION 6 Nov 2024

Comments

1. The discrepancy between the DLS and SEM results regarding the size uniformity of the nanocomposite is notable. While the authors claim a uniform size distribution, the experimental data suggest otherwise. A more detailed analysis of the particle size distribution, potentially using additional characterization techniques, is necessary to reconcile these findings (with ImageJ software).
2. The reported PDI value of 0.37 appears to be inconsistent with the absence of Zetasizer data from the Malvern model. It is unclear how this value was derived, and further clarification on the calculation methodology is required. Additionally, the implications of this PDI value for the nanocomposite's properties and performance should be discussed.
3. The drug release profiles in both acidic and alkaline environments do not appear to have reached a plateau phase. To obtain a more complete understanding of the release kinetics, the authors should consider extending the release study duration.
4. To accurately determine the kinetic behavior of drug release, it is insufficient to rely solely on the R^2 factor. The Akaike Information Criterion (AIC) should be employed to select the most appropriate kinetic model.

Best Regards,

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Pharmaceutics Research Center

Institute of Neuropharmacology

Kerman University of Medical Sciences

Dear Editor-in-Chief,

Thank you for evaluating the manuscript “A novel platform for controlled drug delivery; Green synthesizes and characterization of honey-incorporated Cu/Al layered double hydroxide (LDH) nanocomposite”. We have taken into consideration and addressed all the reviewers’ comments as listed below. The corrected parts were highlighted in Green in the text.

Comments Reviewers 1:

The manuscript, titled "A Novel Platform for Controlled Drug Delivery; Green Synthesizes and Characterization of honey-incorporated Cu/Al layered double hydroxide (LDH) Nanocomposite", lacks the necessary novelty for publication in the Jundishapur Journal of Natural Pharmaceutical Products. This is the primary reason for my recommendation against its publication. Specific comments are as follows:

Query 1: The structure of the article has many grammatical problems and needs to be edited and rewritten.

Reply 1: Thank you for your kind comment; The whole manuscript was edited for typographical errors.

Query 2: The authors conducted a release test at the same pH (7.4 and 7.4) in the abstract section.

Reply 2: Thank you for your kind comment; It was corrected and highlighted in green (Page 2. Line 29).

[In the abstract section] Page 2. Lines 28-29: … and controlled release profile depending on pH (42.3 ± 0.243 % at pH=7.4 and 32.1 ± 0.481% at pH=9.5)…

Query 3: The introduction section is unclear and confusing; the authors do not present why they want to prepare these nanoparticles.

Reply 3: Thank you for your kind comment; the mentioned part was rewritten to show the proposes of the current work (Page 4. Lines 59-78).

[In the introduction section] Page 4. Lines 59-78: In the field of drug delivery, the utilization of inorganic materials may be associated with the production of harmful byproducts and environmental

impacts. Instead, using natural substances and green synthesizing methodology can minimize energy consumption and pollutant production as well as improve human health (18, 19). Accordingly, neutral-derived substances (such as honey) that structurally consist of several organic macromolecules (like carbohydrates, proteins, nucleic acids, and fatty acids) are drawing interest (14, 15). Since natural-based nanocomposite materials are basically considered non-toxic and biocompatible along with high chemical stability and pH-dependent solubility (12, 16). Besides, they are prepared through inexpensive processes and can be easily modified with unique physiochemical properties for different applications such as environmental science, catalysis, biosensing, cosmetics, and medicine (10, 17). Although switching to biological sources might address numerous significant problems, the possibility of rapid degradation of active ingredients during the storage, either by hydrolysis or oxidation, and inadequate therapeutic responses due to the restricted release profiles highlights the necessity of introducing drug delivery systems that can deal with these issues (20). Therefore, the current work was proposed to introduce a new drug delivery platform that can preserve active ingredients from rapid degradation and provide a prolonged release profile by using neutral-derived substances. Accordingly, we aimed to green synthesize and evaluate the physicochemical characteristics of a natural-based nanocomposite using honey as an anionic ligand named honey-incorporated Cu/Al-LDH (Ho@Cu/Al-LDH) nanocomposite.

Query 4: The authors state that a polymer-based LDH nanocomposite film with unique functions and structures can be obtained by incorporating polymers. However, it is unclear why the authors chose honey as the polymer. This choice needs to be justified in the manuscript.

Reply 4: Thank you for your kind comment; proposed comment was noted in the manuscript (Page 4. Lines 59-69, Page 17. Lines 344-348). Hony was chosen mainly due to its high biocompatibility and antioxidant potential as highlighted in the manuscript:

[In the introduction section] Page 4. Lines 59-69: In the field of drug delivery, the utilization of inorganic materials may be associated with the production of harmful byproducts and environmental impacts. Instead, using natural substances and green synthesizing methodology can minimize energy consumption and pollutant production as well as improve human health (18, 19). Accordingly, neutral-derived substances (such as honey) that structurally consist of several organic macromolecules (like carbohydrates, proteins, nucleic acids, and fatty acids) are drawing interest (14, 15). Since natural-based nanocomposite materials are basically considered non-toxic and biocompatible along with high chemical stability and pH-dependent solubility (12, 16). Besides, they are prepared through inexpensive processes and can be easily modified with unique physiochemical properties for different applications such as environmental science, catalysis, biosensing, cosmetics, and medicine (10, 17).

[In the discussion section] Page 18. Lines 362-366: As well, the honey was chosen in this system due to its high antioxidant capacity that can help to preserve the loaded molecules from harsh environments. However, the incorporation of neural-derived substances in delivery systems is mainly considered to be safe and biocompatible together with access for a prolonged release profile.

Query 5: Why did the authors synthesize these nanoparticles? For dye removal, antioxidant properties or drug delivery?

Reply 5: Thank you for your kind comment; the current formulation was primarily synthesized to introduce a new drug delivery platform that can preserve active ingredients from rapid degradation and provide a prolonged release profile by using neutral-derived substances. Following, the dye removal

potential and antioxidant properties of this nanocomposite were also assessed (Page 4. Lines 69-78, Page 16. Line 329-332).

[In the introduction section] Page 4. Lines 69-78: Although switching to biological sources might address numerous significant problems, the possibility of rapid degradation of active ingredients during the storage, either by hydrolysis or oxidation, and inadequate therapeutic responses due to the restricted release profiles highlights the necessity of introducing drug delivery systems that can deal with these issues (20). Therefore, the current work was proposed to introduce a new drug delivery platform that can preserve active ingredients from rapid degradation and provide a prolonged release profile by using neutral-derived substances. Accordingly, we aimed to green synthesize and evaluate the physicochemical characteristics of a natural-based nanocomposite using honey as an anionic ligand named honey-incorporated Cu/Al-LDH (Ho@Cu/Al-LDH) nanocomposite.

[In the results and discussion section] Page 17. Line 347-350: Intercalating drugs into the interlayer spaces of LDH nanoparticles can provide a controlled release profile and improve therapeutic responses while reducing adverse effects. In the current study, we synthesized and evaluated the physicochemical characteristics of Cu/Al-LDH nanoparticles for controlled drug delivery.

[In the results and discussion section] Page 18. Line 352-357: Apart from that, this nanocomposite formulation could be used as an effective and low-cost adsorbent to remove different materials. The removal of pollution, particularly dyes, is an essential step in wastewater treatment and plant health. Besides, Ho@Cu/Al-LDH nanocomposite also revealed good antioxidant properties, which are desirable for preserving oxidation-sensitive molecules during storage.

Query 6: In the results and discussion, the authors said that DLS and SEM results show high uniformity. However, based on the results, nanoparticles aren't very uniform, and in the SEM image, we can see polydispersity.

Reply 6: Thank you for your kind comment; In the DLS technique particles are dispersed in the sample solution, while in the SEM method samples need to be shielded in dry condition. Therfore, the SEM results may seem to be coarsely and flocked. Since particles are dispersed in biological fluids in the body, DLS results are considered more valuable [1-3]. Anyhow, the noted sentence was corrected in the manuscript as **"**Similar to DLS data, SEM micrographs also indicated a restricted range for particle size distribution**"** (Page 11. Line 202 and 203).

[1] Sentis MP, Feltin N, Lambeng N, Lemahieu G, Brambilla G, Meunier G, Chivas-Joly C. Investigation of nanoparticle dispersibility and stability based on TiO2 analysis by SMLS, DLS, and SEM. Journal of Nanoparticle Research. 2024 Mar;26(3):55.

[2] Bootz A, Vogel V, Schubert D, Kreuter J. Comparison of scanning electron microscopy, dynamic light scattering and analytical ultracentrifugation for the sizing of poly (butyl cyanoacrylate) nanoparticles. European journal of pharmaceutics and biopharmaceutics. 2004 Mar 1;57(2):369-75.

[3] Verma P, Maheshwari SK. Preparation of sliver and selenium nanoparticles and its characterization by dynamic light scattering and scanning electron microscopy. Journal of microscopy and ultrastructure. 2018 Oct 1;6(4):182-7.

Query 7: In the release section, the author used a UV spectrophotometer for the release study. The λmax of Ibu is 228. The release study in this λmax with a UV spectrophotometer is inaccurate, and the authors need to use HPLC. How can the authors prove that honey was not released during the release test? Honey

consists of several organic macromolecules that can be released during the experiment. Why did the authors use pH 9.5 for the release test?

Reply 7: Thank you for your kind comment; The potentiality of using UV spectrophotometry for detection of Ibu has reported in literature [4, 5]. Due to the limitations of availability and high cost, HPLC analysis was no used in this study. However, this study is already finished and we aim to use this technique in future studies. Although honey might be released during the release test, the release profile of unloaded formulation (final formulation without Ibu) was also carried out in same conditions as blank to ensure the absence of interaction with other components. The pH 9.5 was used to simulate the intestine condition. Since LDHs becomes substantially tightened at alkaline pHs and might provide a better prolonged release profile (Page 14. Line 278).

[4] Zhou Z, Jiang JQ. Detection of ibuprofen and ciprofloxacin by solid-phase extraction and UV/Vis spectroscopy. Journal of Applied Spectroscopy. 2012 Jul;79:459-64.

[5] Supe KS, Patil JJ. Analytical method development and validation of ibuprofen by UV spectroscopy. World J. Pharmaceut. Res.. 2022 Aug 1;11:1420-30.

Comments Reviewers 2:

Query 1: I just ask you if the project is Affordable or not?

Reply 1: Thank you for your kind comment; The answer to this question needs to be elaborated regarding the availability of key components and scale-up methodology. The current study was conducted as a pilot to assess the feasibility of formulating such a system with existing equipment. However, introducing other logical strategies to scale-up the product could make this delivery system more affordable.

Comments Reviewers 3:

Query 1: The discrepancy between the DLS and SEM results regarding the size uniformity of the nanocomposite is notable. While the authors claim a uniform size distribution, the experimental data suggests otherwise. A more detailed analysis of the particle size distribution, potentially using additional characterization techniques, is necessary to reconcile these findings (with ImageJ software).

Reply 1: Thank you for your kind comment; In the DLS technique particles are dispersed in the sample solution, while in the SEM method samples need to be shielded in dry condition. Therfore, the SEM results may seem to be coarsely and flocked. Since particles are dispersed in biological fluids in the body, DLS results are considered more valuable [1-3]. Anyhow, the noted sentence was corrected in the manuscript as “Similar to DLS data, SEM micrographs also indicated a restricted range for particle size distribution” (Page 11. Line 202 and 203). However, this study has already terminated and we aim to perform the noted analysis in future studies. Besides, the lack of enough experience and difficulties toward access for professional technicians of noted software remain as major issues for recommended analysis.

[1] Sentis MP, Feltin N, Lambeng N, Lemahieu G, Brambilla G, Meunier G, Chivas-Joly C. Investigation of nanoparticle dispersibility and stability based on TiO2 analysis by SMLS, DLS, and SEM. Journal of Nanoparticle Research. 2024 Mar;26(3):55.

[2] Bootz A, Vogel V, Schubert D, Kreuter J. Comparison of scanning electron microscopy, dynamic light scattering and analytical ultracentrifugation for the sizing of poly (butyl cyanoacrylate) nanoparticles. European journal of pharmaceutics and biopharmaceutics. 2004 Mar 1;57(2):369-75.

[3] Verma P, Maheshwari SK. Preparation of sliver and selenium nanoparticles and its characterization by dynamic light scattering and scanning electron microscopy. Journal of microscopy and ultrastructure. 2018 Oct 1;6(4):182-7.

Query 2: The reported PDI value of 0.37 appears to be inconsistent with the absence of Zetasizer data from the Malvern model. It is unclear how this value was derived, and further clarification on the calculation methodology is required. Additionally, the implications of this PDI value for the nanocomposite's properties and performance should be discussed.

Reply 2: Thank you for your kind comment; The DLS method and PDI value were determined by (VASCO®, Cordouan Technologies, France) as corresponding graph and numerical data, respectively. Although the fundamental size distribution generated by DLS is an intensity distribution (D_i), it can be converted to volume (D_v) or number (D_n) distribution using Mie theory [6,7].

[In the results and discussion section] Page 11. Lines 199-203: The PDI is a measure for the size distribution in a given sample. PDI values range from 0 to over 1, where a lower PDI value indicate higher homogeneity. Basically, systems with high degree of homogeneity are anticipated to have a greater prediction chance for physiochemical properties, stability during storage, and release parameters.

[6] Wriedt T. Mie theory: a review. The Mie theory: Basics and applications. 2012 Jun 30:53-71.

[7] Fu Q, Sun W. Mie theory for light scattering by a spherical particle in an absorbing medium. Applied Optics. 2001 Mar 20;40(9):1354-61.

Query 3: The drug release profiles in both acidic and alkaline environments do not appear to have reached a plateau phase. To obtain a more complete understanding of the release kinetics, the authors should consider extending the release study duration.

Reply 3: Thank you for your kind comment; Due to the existed limitations related to equipment and availability of professional resources the release profile study was carried out in predetermined intervals. However, this study is already finished and we aim to perform the noted comment in future studies.

Query 4: To accurately determine the kinetic behavior of drug release, it is insufficient to rely solely on the R^2 factor. The Akaike Information Criterion (AIC) should be employed to select the most appropriate kinetic model.

Reply 4: Thank you for your kind comment; The desired statistic measure, AIC, was conducted to select the most appropriate model. Where, among models with closed R^2 values, the one with a lower AIC value is more reliable. Accordingly, despite the negligible difference between zero order and Korsmeyer-Peppas, the latter associated with a lower AIC value and thus selected as the principal kinetic model in both studied conditions (Page 9. Lines 165-177, Page 16. Lines 311-314, and Table.1 (Page 20. Lines 386-388)).

[In the Materials and methods section] Page 9. Lines 165-177: Further, the Akaike Information Criteria (AIC) was also exploited to test the goodness of fit according to the maximum likelihood using KinetDS software program (KinetDS 3.0). This statistical measure is basically used to assess the applicability of models for a given set of data in terms of their predictive accuracy and relative performance (41, 42). When comparing several models, the one with the minimum AIC value is considered the best fit according to equation 2: $AIC = n \times \ln(WSSR) + 2p$; Equation 2

Where, n is the number of sampling intervals (release data points), p stands for number of parameters (independent variables) in the model, and WSSR is the abbreviation of weighted sum of square of residues, estimated by equation 3:

$$WSSR = \sum_{i=1}^{n} \left[w_i \left(y_i - y_i^{'} \right)^2 \right]$$; Equation 3

Where, w_i is an optional weighing factor, y_i denotes observed value, and y'_i stands for predicted value.

[In the Results and discussion section] Page 16. Lines 311-314: The validity of selected kinetic model also confirmed by AIC values. This mathematical method estimates the prediction errors and helps to assess the best fitting of a model to the data which it was generated from. Therefore, among models with closed R2 values, the model that shows a lower AIC value is more reliable.

Table 1. **Page 20. Lines 386-388:** Results of data fitting parameters and AIC values for different kinetic models in two different pHs (7.4 and 9.5).

	pH=7.4				pH=9.5			
Kinetics model	**R^2**	**AIC**	**K**	**N**	**R^2**	**AIC**	**K**	**N**
Zero-order	0.9868	4.9222	0.1351	-	0.9821	9.1765	0.0914	-
First-order	0.8003	1.7403	0.0017	-	0.8003	2.2838	0.0011	-
Hixson-Crowell	0.9421	8.0947	0.0024	-	0.9365	7.7792	0.0016	-
Higuchi	0.9747	9.5650	2.0534	-	0.9241	9.3043	1.3579	-
Korsmeyer-Peppas	0.9957	4.6872	0.0016	0.9736	0.9832	4.7609	0.0653	0.4161

Best Regards,

A M B,

Pharm D, Ph.D. candidate of pharmaceutics

2 Revision 1

1 comment · 30 Nov 2024

Amanollah Zarei-Ahmady Reviewer

MAJOR REVISION 30 Nov 2024

Dear Author,

I hope this mail finds you well.

Please find my comments and questions on the manuscript "A novel platform for controlled drug delivery; Green synthesizes and characterization of honey-incorporated Cu/Al layered double hydroxide (LDH)" below:

1) Could you please explain the type of carrier you introduced? You introduced it as an anionic carrier!
2) Let me know about possible impurities in honey and their role in synthesis! Also, what about the roles of several organic macromolecules in honey?
3) Do the authors synthesize these nanoparticles for dye removal and drug delivery? The first role wasn't mentioned in the title!
4) Why didn't you analyze the carrier by IR?

I found this manuscript suitable for publication in JJNPP only after major revision.

Best Regards,

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Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Pharmaceutics Research Center

Institute of Neuropharmacology

Kerman University of Medical Sciences

Dear Editor-in-Chief,

Thank you for evaluating the manuscript “A novel platform for controlled drug delivery and dye removal; Green synthesizes and characterization of honey-incorporated Cu/Al layered double hydroxide (LDH) nanocomposite”. We have taken into consideration and addressed all the reviewers’ comments as listed below. The corrected parts were highlighted in Green in the text.

Comments Reviewers 1:

Query 1: The manuscript is valuable and good and seems acceptable.

Reply 1: Thank you for your kind comment.

Comments Reviewers 2:

Query 1: -

Reply 1: Thank you for your kind comment.

Comments Reviewers 3:

Query 1: Please explain about the kind of carrier you introduced? You introduced it as anionic carrier!

Reply 1: Thank you for your kind comment; In this study, a natural-based nanocomposite named honey-incorporated Cu/Al-LDH (Ho@Cu/Al-LDH) nanocomposite was prepared. LDH nanocomposites often utilize ligands to enhance interfacial interactions and stability. Ligands serve as bridging agents that facilitate the anchoring of nanoparticles, metal complexes, onto LDH nanosheets. However, it was corrected and highlighted in the text (Page 5. Line 77).

Page 5. Line 77: “…. a natural-based nanocomposite using honey as interfacial ligand named honey-incorporated …”

Query 2: Let me know about probably impurity of honey and their role in synthesis! Also, what about the roles of several organic macromolecules in honey?

Reply 2: Thank you for your kind comment; The possibility of impurities existence is inevitable. However, the propose of this study was to assess the feasibility of natural substance as alternative source for preparing nanocomposites. Undoubtedly, existence of organic molecules in the process of synthesis might affect meaningfully, which is the main subject of our future studies.

Query 3: The authors synthesize these nanoparticles for dye removal and drug delivery? The first role didn't mention in title!

Reply 3: Thank you for your kind comment; the main propose of this study was to introduce a new platform for controlled drug delivery. Meanwhile, the dye removal potential of this platform was also assessed, offering dual efficacy. However, it was mentioned in the title (Page 1 Line 1).

Query 4: Why you didn't analysis the carrier by IR?

Reply 4: Thank you for your kind comment; The IR analysis measures the interaction of infrared radiation with matter by absorption, emission, or reflection. It is used to study and identify chemical substances or functional groups in solid, liquid, or gaseous forms. While, the HPTLC analysis compare the fingerprint of one sample (unknown) with that of another sample. Accordingly, as we need to assess the production of nanocomposite and existence of honey in the structure, the HPTLC analysis was more appropriate. However, this study is already finished and we propose to run this analysis in future studies.

Best Regards,

Amir Mohammad Bagheri,

Pharm D, Ph.D. candidate of pharmaceutics

3 Revision 2

0 comments

No public comments in this round.