Reviewer 1

The Authors are grateful to the reviewer for their kind suggestions and queries; we addressed most of them.

In this paper the authors present the synthesis of novel gold nanoparticles stabilized with thiolated pectin (SH-PEC) in replacement of the cytotoxic CTAB surfactant used for initial nanoparticles synthesis. The photothermal properties of the resulting NPs were evaluated by irradiating the aqueous suspension of nanoparticles in the NIR range. Moreover, the cell viability assays indicated the increased the biocompatibility of the nanoparticles, and the viability assays with irradiation showed a decrease in the cell viability in comparison to the cells without irradiation.

The results indicated that Au-NPs stabilized with thiolated pectin showed their potentiality in photothermal therapy. However, there are some aspect that should be revised and some additional studies should be done:

1) The authors claim that "there are no reports of AuNRs functionalized with PEC". However there are some recent publications related to Au-nanoparticles coated with PEC and the study of their photothermal applications, especially for photodynamic therapy. The authors should improve the bibliography references and make a brief summarize of the closest examples in bibliography (i.e ACS Omega 2021, 6, 19, 12567–12576, Environmental Science and Pollution Research 2018, 25, 18476–18483 among others…)

Thank you for your suggestion and for the bibliography examples. Absolutely yes, there are many reports in which pectin has been used as stabilizer of plasmonic gold nanoparticular. However, the shape of these plasmonic gold nanoparticles commonly has spherical geometry. Under this consideration, we wrote type in the manuscript "It is important to note that at least in the consulted bibliography by this research group". To enhance the information about it we rewrite the following text:

These stabilization techniques enable precise control over the physicochemical attributes of gold nanorods (AuNRs) while augmenting their functionality. This broadens the spectrum of potential applications for AuNRs, encompassing areas such as biomedicine, electronics, and beyond (Fan, Cheng, & Sun, 2020; Goodwin, 2021; Hakimian & Ghourchian, 2019; Madani et al., 2018; Mahalunkar et al., 2019). Polysaccharides emerges as alternative due to their low cost of production, biocompatibility, and reactivity. Anionic and cationic polysaccharides, such as alginic acid (Figueiredo, Rodrigues, Fernandes, Correia, & Moreira, 2023), , hyaluronic acid (L. S. Li et al., 2021), chitosan (de Barros et al., 2016), gum arabic (Gonçalves et al., 2021, Guzmán-Rocha, Córdova-Fraga, Bernal-Alvarado, & Cano-González, 2022) cellulose (Doghish et al., 2022), starch (You et al., 2020) thiolated dextran (Choi et al., 2010)and PEC (Attallah, Al-Ghobashy, Nebsen, El-Kholy, & Salem, 2018; Cisneros et al., 2021; Nemiwal, Zhang, & Kumar, 2021) have been used to coat and stabilize spherical gold nanoparticles (D. A. de Almeida et al., 2020; Y. Te Liao et al., 2019; Xu et al., 2017; Yoon et al., 2019; Zhang & Lin, 2014). In the present report, pectin (PEC) has been proposed to stabilize gold nanorods. This biopolymer is an anionic heteropolysaccharide, which, like to other biopolymers, has been widely considered in the pharmaceutical area as an encapsulation agent for drugs and active compounds thanks to its solubility, biocompatibility, and biodegradability properties (Martau, Mihai, & Vodnar, 2019,

Kedir, Deresa, & Diriba, 2022). Additionally, PEC has a grafted structure, distinct from other polysaccharides of linear structure used in the stabilization of gold nanoparticles and gold nanorods (Attallah, Al-ghobashy, Nebsen, El-kholy, & Salem, 2018; Beltrán et al., 2022). Furthermore, PEC has been the subject of numerous studies and applications due to its chemical composition facilitates its functionalization or chemical modification with different types of molecules (Borker & Pokharkar, 2018; Cisneros et al., 2021; D. qiang Li et al., 2021). It is important to note that at least in the consulted bibliography by this research group, there are no reports in which the PEC was used to stabilize AuNRs.

2) It is recommended to improve the section Materials and Methods. The information concerning different techniques should be improved. For example, the characteristics of potentiometer (reference electrode, salt bridge, analyte and an indicator electrode). Instrument specifications for FT-IR, the information about obtaining calibration curve in ICP-MS and reference standards used, etc…

Thank you for the recommendations. The information about each one of the instruments was added in the respective section.

3) There are some additional characterizations missing to have a close information about SH-PEC obtaining and purification. In fact there is not a characterization of SH-PEC purity. The authors should must to perform chemical analysis and NMR analysis to ensure the obtaining of the final ligand and the purity of final product. The raw data can be detailed in supplementary information.

Thanks for the comment. Indeed, it is true that completely removing of CTAB molecules form aqueous medium is practically impossible. We would like to give highlights about the strategy followed to get AuNR-CTAB and AuNR@SH-PEC. Firstly, the AuNRs were synthesized using a 0.1 M solution of CTAB. After the growth process and get the gold nanorods, these were centrifuged and resuspended in a 10 mM CTAB solution to keep them in suspension until the coating process with SH-PEC. It is important to note, that AuNRs in 10 mM of CTAB solution, the shape of AuNR remains preserved. After the SH-PEC polymer coating process, an exhaustive washing process was conducted. This involved three washing with water (mili-Q) by centrifugation to ensure the majority of CTAB molecules were removed from the solution. Furthermore, the observed charge inversion in zeta potential measurements and the enhanced cell viability suggest that the amount of CTAB molecules remaining in the solution is negligible.

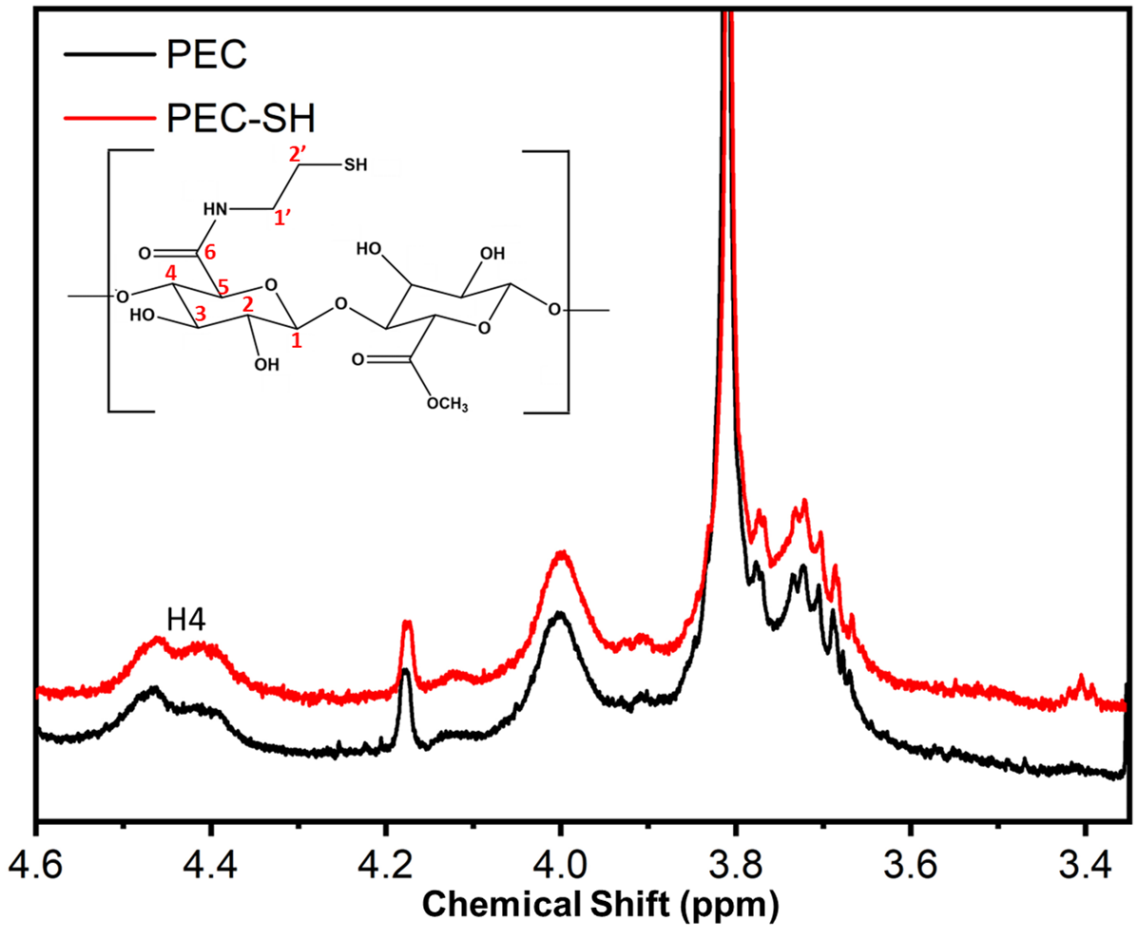
Additionally, NMR analysis was used to determine the substitution degree of PEC. The information of the experiment set up was added to Materials and Methods section.

2.5 Nuclear Magnetic Resonance (^{1}H NMR)

The degree of substitution (DD) of PEC by cysteamine was determined using ^{1}H NMR spectroscopy. ^{1}H NMR spectra was recorded using a Bruker DRX-500 spectrometer (Avance, 400 MHz). For the analysis, both PEC and SH-PEC solutions were prepared at the concentration of 1 mg/mL using D_2O as solvent. The NMR spectra were recorded at 300 K, using tetramethyl silane as reference. The DS of SH-PEC was calculated by comparing the signal area of the H1′ of cysteamine (3.40 ppm) to the area signal area of H4 (4.40 ppm) corresponding to the galacturonic acid residues (E. A. M. S. Almeida et al., 2015):

$$DS = \frac{H1'(3.40)}{2H4(4.40)} x100\%$$

Figure 3 shows the ^{1}H NMR spectra recorded for PEC (black line) and SH-PEC (red line). A complex set of signals located at 3.78 to 3.65 ppm, characteristic peaks showed by native PEC, however, signals correspondingly to esterified methoxyl groups and H_2 of galacturonic residues appeared at 3.77 and 3.70 ppm, respectively (E. A. M. S. Almeida et al., 2015). The signal showed as double peak between 4.45-4.35 ppm can be assigned to the H4 of galacturonic acid (4.40 ppm) and esterified galacturonic acid residues (4.46 ppm) (Hong, Zhao, Yin, Nie, & Xie, 2022). The triplet signal appeared at 3.40 ppm in the spectrum recorded for SH-PEC, correspond to the protons of cysteamine (tagged as H1') (Madison Metabolomics Consortium - Qiu Cui, Ian Lewis, Francisca Jofre, Mark E. Anderson, John L. Markley, Biological magnetic data bank doi:10.13018/BMSE000388, https://doi.org/10.1039/C5TX00090D). Then, the substitution degree of PEC was determined considering the area ratios of H1'/H4(4.40 ppm) (E. A. M. S. Almeida et al., 2015), resulting in a 6.9%.



4) DLS and z-potential spectra and recorded conditions should be showed in supplementary material.

In attention to the suggestion given by the reviewer, the information and graphs were added in the supplementary information.

5) In the same way ICP-MS of the final NPs should give information concerning the % of inorganic-organic part and could help to determine exactly the number of HS-PEC molecules per particle. The raw data can be detailed in supplementary information.

This is an interesting suggestion. However, we regret that we cannot address this observation. The challenge arises because we did not dry the AuNR@SH-PEC to obtain its total mass. This step is a problem since after the lyophilization process, the lyophilized material is stuck to the wall vial, moreover, the remaining mass is very little weigh accurately.

6) Could the authors correlate the UV-Vis bands shifts with size?. It would be interesting correlate the surface coating and possible aggregation processes with plasmon resonance.

This is other interesting suggestion. Probably, it needs a more exhaustive analysis to do this task. In this study, we used the UV-Vis spectroscopy as control quality tool and, thus, to determine the quality of the AuNR-CTAB and to follow the coating process of AuNR@SH-PEC, because is a facile to use and quick analysis tool.

7) Although production of ROS is not high, it can be considered the influence of increased ROS production (10-50% depending on the coating) in the cytotoxic effect of the nanoparticles. In fact, if we observe the cytotoxic effect in different cell lines there is a notable difference between fibroblast and HeLa cells that could be related to this effect since the ROS sensitivity of different cell lines can be dissimilar. Please, could the authors give some explanation in this sense?

In attention to this suggestion the Authors rewrite this section as:

Reactive oxygen species (ROS) play a pivotal role invarious cellullar funtions, including differentiation and the killing of cancerous cells (Villalpando-Rodriguez & Gibson, 2021; Zaidieh, Smith, Ball, & An, 2019). In this context, the ROS production in Balb/c 3t3 and HeLa cells stimulated by the presence of AuNR@SH-PECs during the laser irradiation process was studied (Figure 11). In accordance with Figure 11, the irradiation of Balb/c 3t3 and HeLa cells with the 808 nm laser in the presence of AuNR@SH-PECs resulted in a slight incrase in ROS production compraed to the control cells (those laser irradiated without AuNR@SH-PECs). Specifically, Balb/c 3t3 cells showed a 47 % increase in ROS production when they were incubated with AuNR-@SH-PECs stabilized with 8 mg/mL. In contrast, HeLa cells reached peak ROS production with AuNR-@SH-PECs stabilized with 4 mg/mL. his differential response can be attributed to the heightened metabolic activity inherent to cancer cells (Villalpando-Rodriguez & Gibson, 2021; Zaidieh, Smith, Ball, & An, 2019).

Concerning the use of Pectin, it should be recommendable that the authors highlighted the biological implications of use Pectin, apart the benefits of being a biocompatible material (ref: International Journal of Biological Macromolecules, 185, 2021, 49-65)

In attention to this suggestion, highlighted information on the characteristics and properties of the PEC was added:

Additionally, PEC has a grafted structure, distinct from other polysaccharides of linear structure used in the stabilization of gold nanoparticles and gold nanorods (Attallah, Al-ghobashy, Nebsen, El-kholy, & Salem, 2018; Beltrán et al., 2022). Furthermore, PEC has been the subject of numerous studies and applications due to its chemical composition facilitates its functionalization or chemical modification with different types of molecules (Borker & Pokharkar, 2018; Cisneros et al., 2021; D. qiang Li et al., 2021). It is important to note that at least in the consulted bibliography by this research group, there are no reports in which the PEC was used to stabilize AuNRs.

8) Other minor changes:

Last word in Abstract: change therapist by therapy.

The Authors corrected this typo error.

In general terms, there is a notable piece of work to be published in Pharmaceutics. However, different aspects summarized in the present report should be addressed before acceptation for publication.