**Response to Reviewers comments**

**Manuscript title:** Surface Modification and Functionalization of Sorafenib-Loaded PLGA  
Nanoparticles for Targeting Hepatocellular and Renal Cell Carcinoma  
Manuscript ID: pharmaceuticals-2580103

The point to point response has been made to the reviewers’ comments. The changes have been incorporated in the manuscript and were highlighted.

**Reviewer 1**

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| **S/No** | **Comments** | **Response** |
| 01. | Would you please explain how the SEM confirmed remained stable for a longer period as you describe | Scanning Electron Microscopy (SEM) is basically performed for the size and morphology of nanoparticles. So if the size and morphology before and after freeze drying is the same, it means nanoparticles are stable. |
| 02. | Would you please label what is the SPF1 to SPF10 in this article? Or you can directly label them in the table1 | SPF (S=for Sorafenib, P=PLGA and F= Pluronic F-127), so it was shortened for ease and convenience as per practice. While 1 to 8 shows simply the numbering, means formulation 1, 2, 3…. and 8, due to different drug to polymer ratios. |
| 03. | In Figure 3 would you please combine the drug concentration in different major organs with different formulations | It is measure of radioactivity and The radioactivity are always given in percentages. The percent radioactivity shows the availability of drug in that particular organ. The Figure is 7 in revised manuscript. |
| 04. | This article's workload is a bit less. Would you please add some cell experiments | The authors are of the opinion that the data is sufficient for an article to be published in a journal. |

**Reviewer 2**

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| S/No | Comments | Response |
| 1 | The title speaks about surface modification and functionalization for targeting purposes and yet the functionalization is not discussed in the manuscript. Pluronic f-127 was used as a stabilizer. In the abstract it is referred to as coating. Therefore the title, abstract and text body become ambiguous | Pluronic (F-68 and F-127) acts as stabilizer, surfactant, permeation enhancer (blocking efflux transport protein system, pgp) and also as coating material when used in formulations. As it is an amphiphilic (having both hydrophilic and lipophilic portion) polymer, so it can be easily attached to the surface of nanoparticles and thus can alter the surface nature of the particles. It has a role in physical targeting of nanoparticles and this property is due to the fact that it can camouflage the particles by avoiding the reticuloendothelial system (RES) of the body (Functionalization). So Pluronic have a broad spectrum of use in nanoformulations. |
| 2 | The Introduction is fragmented and the background of the study is not fluently presented. The aim of the study should be clearly stated at the end of the introduction. Furthermore, a better provided rationale and novelty of the current experiments is needed as in the literature data about PLGA nanoparticles with sorafenib can be found (https://doi.org/10.1016/j.jconrel.2015.11.003 ; https://doi.org/10.3390/polym10080895 ; 10.2147/IJN.S415968) | Thanks you very much for the valuable suggestion. Introduction was set as per the suggestion of the reviewer. The aim of the study was incorporated in the last paragraph. The paper in reference helped the author to correct and organize the introduction section. |
| 3 | Section 4.3.7. Formulation optimization is entirely missing from the manuscript | Formulation optimization portion has been added in both material and method and Results section. |
| 4 | In Materials Tween 80 is mentioned, but not used anywhere in the experiments | It was a typographical mistake which has been rectified. |
| 5 | Clear coding and composition of the investigated batches is missing. What are SPF1, SPF2, etc | It was added to materials and methods and results section of the manuscript. |
| 6 | Why in some experiments SPF1, SPF2 and SPF4 are investigated while in others SPF1, SPF2 and SPF3? There is no explanation why the rest are entire left out | It was a typographical mistake which has been rectified. The optimized nanoformulations are SPF1, SPF2 and SPF4. |
| 7 | Different apparats are given in 4.1. Chemicals and equipment and 4.3.2 FT-IR analysis. Which one is correct? Furthermore, either place all the equipment in the section 4.1 or provide them at the corresponding place in the methods section. Please, do not mix the two ways as it makes the information difficult to follow or repeat by other researchers | The FTIR analysis was discussed in the manuscript, due to the fact that it was used to carry out the compatibility of the drug and polymer with other excipients used in the formulations. |
| 8 | Figure 1 and 3 combine too much information and there is no clear labeling on what all those different images represent (e.g. figure 1 has 3 times A, B, C, D which are not identified). The quality could be improved as well | The figures were separated and corrected as per instructions. The quality has also been improved. |
| 9 | Figure 3 shows the release from Pluronic f-127. What is it supposed to mean? | It has been corrected to the “ the release of sorafenib from the PLGA nanoparticles using Pluronic F127 as a stabilizer. |
| 10 | There are some statements which do not coincide with the presented results: p. 3 lines 103-104 "the increase in SFB concentration was directly proportional to the nanoparticles size". Table 1 shows different results. | These statements were corrected accordingly. |
| 11 | It is unclear whether the data is correct, especially for the particle size. Different data sets provide different results for it- Table 1, Figure 1 regarding the particle size and the SEM images | The data in table is correct and in the graph only one reading i.e., 1792 was mistyped to 792, which was corrected |
| 12 | Why there is no data about the particle size, PDI and zeta of the placebo nanoparticles? The size of "plain nanoparticles" appears only in the abstract. It is nowhere else shown neither it is discussed why the plain nanoparticles are bigger than the "coated" ones | Thanks for the correction. The data was added in table 1 as suggested. |
| 13 | On p. 10 line 238-241 the authors state that "The %EE has not been altered significantly by changing the ratio of SFB to polymer". Yet, in Table 1 the %EE ranges from 60-99%. | The sentence is rephrased. |
| 14 | The authors speak about the influence of "various emulsifiers" (p. 10, line 258) and "different stabilizing agents" (p. 14, line 390). Yet, in the text information only about Pluronic f-127 in 1% concentration can be found. It is misleading in its current state | There was only one stabilizer used in this particular study. The typographic mistake was corrected to “different concentrations of drug”. |
| 15 | Where is the data about the control solution (p.6 line 194-195)? It cannot be seen neither in Table 3 (probably the data for reference?), nor in Table 4 or Figure 3 (which image). | The control solutions (Sorafenib Nanosuspension without PLGA and pluronic F127) were used to compare the *in-vivo* pharmacokinetic parameters of polymeric nanoparticles with control solutions of sorafenib as no conventional iv preparation of the drug are available. |
| 16 | The authors state "Distribution of SFB nanoparticles formulation was higher in liver as compared to control solution due their smaller size" which cannot be the reason as a solution doesn't contain any particles. Please provide better explanation | The control solution is actually sorafenib Nanosuspension without PLGA and pluronic F127. So its particle size is higher as compared to polymeric nanoparticles using pluronic F 127 as a stabilizer. While the polymeric nanoparticles are smaller enough in size for physical targeting of liver and kidneys. |
| 17 | The explanation of the in vivo drug analysis is unclear. What does the control solution contain? How was its distribution determined (was it also labeled and how)? | Here in this study the word control solution means plain sorafenib Nanosuspension in sterile water having the particle size of ˃200 nm. It is used as stock while the dilutions are prepared as per nanoformulations concentrations.  Sorafenib solutions (Nanosuspension) and nano-formulations (with PLGA) were directly loaded with radionuclide from the TC 99m generator using stannous chloride as a reducing agent. To 50 µl of stannous chloride solution (2 mg/ml) 1 ml from both sorafenib solution and nanoparticle formulation were added and the pH was maintained to 7.0 (6.0-8.0) with sodium hydrogen carbonate solution (0.5 M). The prepared solutions were then incubated for 15 min, individually with freshly prepared at room temperature. The radioactivity of the solutions was finally found out by dose calibrator. All the parameters such as; the amount of stannous chloride, pH of the preparation, and the incubation time were validated and optimized for the labeling efficiency |
| 18 | What does "animals with any discomfort were excluded from the study" mean? Discomfort due to what? What is defined as discomfort? | Acclimation and stabilization of experimental animals is a part of the *in-vivo* study as if there is any discomfort (salivation, screaming, tremor, restlessness, dyspnea, diarrhea, or state of coma) in animals, it will ultimately affect the results. The discomfort may be due to any factor i.e., pain, fever or any other experimental condition. That’s why at least a week time is recommended for the rabbits for acclimation. |
| 19 | Why the entrapment efficiency was determined by centrifugation at 30oC while in the preparation section the centrifugation was at 4oC? Which supernatant was investigated to evaluate the "free drug in the supernatant"? | It’s a typographic mistake and has been corrected; centrifugation was carried out at 4 °C. After centrifugation of the nanoformulation, the supernatant was analyzed by UV-Spectrophotometer for the free drug as the settled part is nanoformulation. |
| 20 | The conclusion section requires complete rewriting because currently it doesn't show the actual findings of the presented research. | The conclusion section is rephrased as recommended. |