

**Ms. Ref. No:** IJPHARM-D-22-02441

**Title:** Oral empagliflozin-loaded tri-layer polymeric fibers fabricated using tri-axial electrospinning: Enhanced in vitro and in vivo antidiabetic performance

**Journal:** International Journal of Pharmaceutics

**Response to Reviewers:**

**Reviewer #1:**

**Question 1:** The 4th and 5th paragraphs about nanotechnologies and electrospinning make no sense. To project the merits of your job, several sentences about the developments of electrospinning from the single-fluid process (10.1016/j.ijpharm.2022.121558; 10.1016/j.ijpharm.2022.121510), to coaxial (https://doi.org/10.3390/membranes11110802; 10.1016/j.ijpharm.2022.121809), side-by-side (https://doi.org/10.3390/pharmaceutics14061208), triaxial (10.1016/j.jallcom.2020.156471), and tri-layer side-by-side (https://doi.org/10.1007/s42114-022-00478-3) for creating monolithic (10.1016/j.ijpharm.2022.121513), core-shell (DOI:10.1007/s42114-022-00551-x), tri-layer core-shell (10.1016/j.cej.2021.131455), and tri-layer Janus nanofibers (https://doi.org/10.1007/s42114-022-00478-3). These electrospun nanostructures provide more and more strategies for developing new kinds of medicated nanomaterials and nano DDS (10.1016/j.ijpharm.2022.121888; https://doi.org/10.3390/polym14091637; https://doi.org/10.3390/polym14112132). Among them, tri-layer core-sheath nanofibers show great potentials for furnishing an improved drug sustained release profile (https://doi.org/10.3390/biom12081057; https://doi.org/10.1186/s12951-022-01463-0).

**Response 1:** Firstly, we very much appreciate your supportive comments. The 4th and 5th paragraphs in the "Introduction" of the manuscript [have been modified to convey clear message](#).

**Question 2:** If possible, TEM images should be provided to show the tri-layer core-sheath nanostructures.

**Response 2:** Unfortunately, we can not perform the TEM analysis because of the insufficient budget. There is no TEM in our laboratory and the service procurement of TEM is so expensive in our country. Instead of this, it was added one more FIB-SEM image of the tri-layer core-sheath nanofibers. These are the best FIB-SEM images we have.

**Question 3:** What is the solubility of empagliflozin in water and also the organic solvents.

**Response 3:** Empagliflozin is very slightly soluble in ethanol (8 mg/mL), sparingly soluble in methanol (33.5 mg/mL), slightly soluble in acetonitrile (2.6 mg/L), slightly soluble in 50% methanol in water (6.4 mg/mL), soluble in 50% acetonitrile in water (68 mg/mL), practically insoluble in toluene, and very slightly soluble in water (Gundle et al.; Niguram et al., 2020). Therefore, chloroform/methanol (3/1, v/v) mixture was prepared for producing

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fibers due to chloroform being the solvent of PCL and methanol being the solvent of empagliflozin.

Gundle, M., Danao, K., Shivhare, R., Rokde, V., Warokar, A., Mahapatra, D., Methodical insights into reported sophisticated analytical techniques for the determination of anti-diabetic drug empagliflozin in various pharmaceutical products.

Niguram, P., Polaka, S.N., Rathod, R., Kalia, K., Kate, A.S., 2020. Update on compatibility assessment of empagliflozin with the selected pharmaceutical excipients employed in solid dosage forms by thermal, spectroscopic and chromatographic techniques. *Drug Development and Industrial Pharmacy* 46, 209-218.

**Question 4:** How about the double-layer core-shell nanofibers for encapsulating empagliflozin? The main advantages of the additional outer layer please be discussed to compare with a blended shell layer of the PMMA and PLA.

**Response 4:** [further clarification on additional layer is now provided in Discussion section](#), as below:

"In this study, the main aim is to protect empagliflozin from the acidic environment of gastric juice and provide the controlled and sustained release of the drug, thus reducing the frequency of drug dosage, improving patient compliance, and increasing the bioavailability of drug compared to empagliflozin powder form. Also, the drug release test demonstrated that 97% of empagliflozin was released in 24h. If we make only two-layer it would be released faster and we need to repeat the dose in 24h, but our target is giving the drug once daily. Consequently, having more layers provides more protection, an extended half-life, and increased degradation time."

**Question 5:** Is it possible that empagliflozin presented in the inner core in a nano crystal state and exert negative influence on the drug release and therapeutic effects.

**Response 5:** It doesn't seem so. In this study, *in vitro* drug release tests and pharmacokinetic test were performed for the evaluation of therapeutic effect and release profile of the drug. It was found that all of the empagliflozin-loaded nanofibers demonstrated a burst drug release by releasing 42.8% in the first 30 min. Furthermore, empagliflozin contained in fibers was released in a controlled manner in 48 h. In pharmacokinetic evaluation, empagliflozin powder lost its blood glucose lowering effect in 24 h while empagliflozin-loaded nanofiber showed a longer blood glucose lowering effect by its sustained release profiles for 48 h. Furthermore, empagliflozin-loaded nanofiber exhibited a longer release time due to its strong degradation mechanism caused by the hydrophobic character and tri-layer structure of PCL/PLA/PMMA nanofiber. Therefore, the positive advantages of empagliflozin-loaded nanofiber on the drug release profile and therapeutic effects compared to empagliflozin powder were proven by performing *in vitro* drug release tests and *in vivo* animal tests. [Data shows](#), no negative influence of the crystal state of empagliflozin on drug release.

**Question 6:** References please be updated. The pioneer jobs from Prof. Edirisinghe's group in UCL is very very important. But this does not prevent you to discuss your studies with the most recent developments in this field, which should benefit a higher impact of your article after publication.

**Response 6:** Firstly, thank you for your supportive comments. The manuscript was improved by adding new references [and](#) highlighted in the [manuscript](#).

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Reviewer #2:

**Question 1: In vitro drug release profile of EM-loaded PCL/PLA/PMMA fiber in solution simulating stomach acid should be added.**

**Response 1:** Firstly, we very much appreciate your supportive comments. Normally, *in vitro* drug release test was performed by simulating stomach acid. However, there was a mistake in the manuscript. This is now corrected. Thank you.

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**Question 2. The mass weight of the nanofibers used in the cell proliferation assay should be described in the manuscript. The cell proliferation assay should be done with different mass weights of nanofibers.**

**Response 2:** We understand the reviewer's concerns and recognize the importance to observe the effect of the samples of different sizes on the cells. For this study, a single size (5 mm in diameter) fiber sample was chosen so that the sample sizes could be standardized in the studies. This size was chosen to be compatible with the 96-well culture plate. The effective dose and pharmacological analyzes of the drug-active substance used were previously performed. Since loading efficiency and release properties were characterized in this study, different sizes or numbers of drug-loaded fibers were not analyzed. The aim of cell studies was to find out any (cytotoxic or metabolic) effect of the empagliflozin-loaded tri-layer polymeric fibers on cells. The fiber size-cell number ratio used in the studies remains high when compared to *in vivo* studies and increasing this ratio will cause it to be incompatible with the physiological conditions that can be observed *in vivo*. Different drug dosage applications are expected to have different levels of effects on cells and tissues. For this reason, it was not preferred to increase the fiber number in order not to observe the effects of an overdose that would not be clinically compatible.

**Question 3. The equation of BAR of nanofibers after oral administration is wrong, and it should be corrected.**

**Response 3:** It is now corrected.

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**Question 4. For in vivo studies, the dose of EMF was 5 mg/kg. Is it the dose of EMF total or the EM in EMF?**

**Response 4:** Firstly, the encapsulation efficiency of empagliflozin in EMF was calculated. According to the result of the encapsulation efficiency, the dose of EMF was arranged as 5 mg/kg like EM powder for the oral administration in *in vivo* animal tests.

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**Question 5. Some experiments should be added to evaluate the biocompatibility of nanofibers in vivo. For instance, hematoxylin-eosin staining of stomach...**

**Response 5:** Hematoxylin and eosin staining was applied to perform the morphological evaluation on the liver, pancreas, and kidney tissues for histological analysis. Unfortunately, hematoxylin and eosin staining can not be done on the stomach because stomachs were not taken after decapitation.

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**Question 6. Please check the abbreviations throughout the manuscript carefully. Some words with abbreviations appeared twice or more. For instance, Poly(L-lactic acid) (PLA), Polycaprolactone (PCL) ...**

**Response 6:** It was revised according to the reviewer's suggestion. Thank you for your attention.

**Question 7. Both FTIR and FT-IR appeared in the manuscript, and it should be unified.**

**Response 7:** It was revised according to the reviewer's suggestion.

**Question 8. The tense in the manuscript should be carefully checked for consistency.**

**Response 8:** Proofreading was done in the revision step by a native speaker.

**Question 9. In the equations,  $W_0$  should be changed.**

**Response 9:** It was corrected.

