Answer to the review of Prof Éva Kiss

Author's gratitude: *The author is grateful to the reviewer Prof. Kiss Éva for her valuable suggestions and appreciable remarks. Despite of busy schedule, the reviewer has kindly finished the reviewing within very short time, gave significantly important suggestions to improve my thesis and to correct my mistakes and I am highly thankful for that.*

General evaluation

The form of the Thesis corresponds to the generally accepted structure with clear chain of ideas and definite description of the aim of the research. The *Introduction* with critical *Review* of previous works is followed by *Experimental part, Results and Discussion,* as well as *Conclusions*. The demonstration of the subject and the own experiments are sufficiently detailed and appropriate with suitable Figures and Tables. The results in generally are presented in a proper way. (Especially the Pareto charts are informative.)

The *New Scientific Results* of the research work are summarized in 5 thesis points in a rather concise way. It is reasonable in most cases. In the case of point 4 however, the present form is so general, that it does not contain new results.

I accept the thesis points as new scientific results except the thesis number 4. (For that I suggest to include the results obtained for and statements related to the systems studied by the author.)

Answer: *I must agree with the reviewer and it is a pity that I cannot change it in this stage. First verion of my submitted thesis had extended version of thesis points and reviewer had no comment on them. In my revised thesis, I tried to make thesis points more brief and attractive based on the suggestion given by a professor during my home defense. Unfortunately I made thesis point 4 very general and it does not show clearly our new results. The only subterfuge is that the time was very little for correction, and I was not precise enough. Nevertheless, I have two published papers cited for the reviewer in connection with that thesis point which can be considered to forgive my writing mistake.*

Remarks and comments

I have some comments relating the introduction of the subject, the experiments and their evaluation.

1. p. 19 "In a physiological medium, extremely hydrophilic macromolecules like dextran or poly(ethylene glycol) (PEG) are often used to stabilize colloidal particles, since they interact strongly with the particle surface, and that they cannot form 'bridges' between different particles." I argue that statement, there are examples for bridging flocculation by PEO polymers, since that phenomenon is dependent on the surface coverage of the particles by the adsorbing polymer as well as on the molecular weight but not on the type of polymers.

Answer: *There is no doubt that the statement is debatable from the point of view of the reviewer. In some cases bridges might form. However, as I know when the steric force is the main force for stabilizing colloidal particles using polymer coatings, bridge formation is unexpected.*

2. The *encapsulation efficiency* is a central question. Application of a direct method to determine the drug content is more convincing in my opinion.

Answer: *With due respect to the reviewer's opinion, it can be mentioned that our indirect method gave very precise result, and hence direct method was not obligatory to be sure about our result. Previously our research group has published an article showing that direct and indirect methods needed for our case gave similar result, whereas direct method was quite complicated. In some cases e.g. drug attachment on the particle surface instead of microencapsulation, direct and indirect methods can show significantly different results which is not our case.*

3. The *coencapsulation* of the magnetic nanoparticle and the bioactive component is also a crucial achievement. It would have been nice to see the experimental evidences collected that the two types of particles are present together in the PLGA carriers.

Answer: *The encapsulation efficiency of active material was measured indirectly and TEM image showed the encapsulation of magnetite. I agree that some direct measurements would have confirmed the achievement more strongly.*

4. p. 60. There is a good example to show what the benefit of the experimental design is to explain the common effect of PLGA and HSA concentrations.

Answer: *The figure on the mentioned page is a really good example to show the benefit of experimental design. In the thesis, it was discussed. Maybe the discussion was insufficient from reviewer's point of view. However, it can be assured to the reviewer that we are aware of the benefits of experimental designs.*

5. On the surface modification of the PLGA particles by Pluronic 68
Since the surface of the PLGA nanoparticles might contain a certain amount of PVA the evaluation of the composition of surface layer and its influence on physico-chemical properties is not easy following the Pluronic adsorption. This can lead to surprising and sometimes confusing experimental findings. The discussion of these data is moderate and appropriate in the thesis work.

Answer: *I completely agree with the reviewer. Complete removal of PVA emulsifier from nanoparticle surface prior to redispersion was not possible and also not found in any published work done by others. It is a pleasure for us that the reviewer has found our discussion moderate and appropriate.*

6. Particle size distribution curves in Figure 23 present a wide size distribution which does not allow us to exclude the possible aggregation.

Answer: *If we compare the size distribution curves of unmodified and modified particles, we can see that poloxamer addition did not change the distribution trend. In my opinion aggregation could have been observed if another peak had been appeared in the bigger size regions.*

Questions

1. What can be the role of position of shear plane in the interpretation and comparison of measured zeta potential data?

Answer: *Zeta potential could be drawn by an exponentially decreasing curve from the surface of a particle to the solution. Thus, the zeta potential is higher if it is measured close to the particle surface which means it is highly dependent on the shear. The higher the shear, the higher the measured zeta potential. The shear surface depends on the viscosity which was determined for the studied dispersions, and the Zetasizer instrument calculates with these values.*

2. How do you see the possible future application of the drug delivery system developed?

Answer: *Since my PhD work was on drug development system, I am very hopeful to see revolutionary change in the future and also want to work as an active researcher for that.*
This system has been developed for curing hepatitis, however, it has high potential in the fight against hepatocellular carcinoma. For this latter purpose it is necessary to reach the cancer cells efficiently, for which its surface should be functionalized by a suitable targeting agent. The research group is intent to improve this composition and is searching for partners who can contribute to test it in in vivo studies.

Veszprém, 23 September, 2014

Quazi Tanminul Haque Shubhra