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UNIVERSIDADE DE COIMBRA

Report of the Ph.D Thesis submitted by Michaela Kohlová, Mgr

Mgr. Michaela Kohlová from the Department of Analytical Chemistry of the Faculty of Pharmacy in Hradec Králové present the doctoral thesis *"DEVELOPMENT OF NEW TYPES OF BIOCOMPATIBLE HAEMODIALYSIS MEMBRANES FOR SEPARATION OF BIOMOLECULES*" to get a Ph.D. degree at the Faculty of Pharmacy of Charles University, on a bilateral agreement between the Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic and Faculty of Pharmacy of University of Porto, Portugal.

There is a growing concern on the part of the scientific community in terms of Public Health intervention, improving the quality of life of patients undergoing haemodialysis treatment in order to minimize health risks associated.

This dissertation awakens the importance and the implications on this research area. The development of dialysis membranes (HD) is of the greatest importance, and this dissertation brings a valuable analytical contribution.
This dissertation contributes to answer to some of the questions that are placed in the introduction and the thesis develops a research work in a very important and innovative aspect.

Overall, the thesis is well structured, appropriate with respect to the objectives identified and questions enquired. It is well written and the candidate demonstrates a solid understanding of the state of the art in the research area and the knowledge of the most important and current scientific literature. Aims and methods are clearly described. Michaela Kohlová represents the ideas and knowledge with sufficient theoretical background. I really enjoyed reading this thesis.

The thesis, with 191 pages all together, is elaborated in different chapters. The candidate presents the structure of her dissertation divided into 3 main parts: the first theoretical, a second one where she presents the experimental work developed and last, the concluding remarks.

Global analysis of the thesis presented:

1- The objectives of the thesis are concise and real.

2- The theoretical background of the research is both well-structured and written. The objectives it proposes are reached, supported by a review of the current scientific bibliography on the subject, a total of 270 references. The candidate has studied and used an appropriate number of bibliographic sources cited in the thesis.
The theoretical introduction and the necessity of minimizing complications of ESRD patients by improving the haemodialysis membranes (chapter 3.5) and the learning from the point of view of different types of membrane preparation and characterization (chapter 3.5 to 3.10) are pointed, as well as the author's ability to perform the scientific work at the required level.

3- Chapter 4 is the core of the research of the present thesis. The aims, research design, background of the research problems, hypothesis and research methodologies are clearly described and fully justified in this chapter, as so the results of research and its interpretations and conclusions.
The chapter 4 shows the active research work developed by Michaela Kohlová in the pursuit of improvements related to the biocompatibility and bioactivity of HD membranes.
It gives evidence of the deep theoretical knowledge and excellent focus of the problem examined in the thesis.
The results are clearly presented, with the appropriate controls and statistical analysis. Moreover, results are compared in relation to the research of other works published in the scientific literature, and the thesis demonstrate a good understanding of the implications of the work in a broader scientific context.
The results of this thesis and their benefit are not prejudiced by the preliminary results of own research, but open to alternative approaches.

4- In section 5.9 Michaela Kohlová presents a summary of the conclusions of the whole work and presents the future perspectives.

The research in the development of innovative haemodialysis membranes generates significant new scientific knowledge as demonstrated by the publications in international journals of high standard, where Michaela Kohlová is the first author, showing the candidate's independent contribution to the research.
I highly appreciated this element, because it means that the most substantial part of the candidate's results were subjected to an independent peer-review process which is the proof of its quality.

My comments outlined above do not call for major changes, offers as unequivocal recommendation of the acceptance of the thesis and the permission for a public defense of the dissertation is granted.

Although the thesis fulfils the formal requests on a very good level.
I have the following minor comments:

1- Relative centrifuge strength should be given in *g* as mentioned in page 128 and 129 and not in *rpm* as referred in pages 141 and 165. Besides the centrifuge is not included in the instrumentation in section 4.1.2.2.

2- Some abbreviations are missing in Index of abbreviations for example:

- *g* and *rpm* are well known, but should be included.

- URR is missing

- PMNs is mentioned in page 152, but in the index of abbreviations is indicated PMNLs.

3- Pag 158: LC-MS-MS conditions and validation data should be briefly described, or the bibliographic reference of a published work where these data are described, should be introduced.

4- Legend of figures: is not necessary to repeat in all legends the full name of the abbreviations. For example: α-TCP, ALA, NMP, PSf.

5- The legends of the tables are too long and should be more succinct. For example, in table 13, page 142, some information should be mentioned in the text. Other kind of information is repeated.

Moreover, once again the full name of the abbreviations is included. For example: α -TCP, ALA, NMP, PSf, AA, LPO and TAS.

For the public discussion I have the following questions:

1- Pag 135- The concentration of α-TCP was fixed at the lower concentration of 5 mg/mL in NMP since higher concentrations negatively affected URR values. Why did you not test a lower concentration?

2- Pag 137- In chapter 4.2 "Development of bioactive membranes by incorporation of antioxidants". The mixture of α -TCP and ALA increase the antioxidant activity of the membranes as shown in fig 34. Since the work and the results presented are based on the use of a homemade bioactive HD membrane, inter-day precision evaluation is crucial. Inter-day precision was evaluated? It is not clearly stated in the text.

3- In section 4.2.7, page 140 last paragraph the authors mentioned "… *the modified membranes were used for experiments within one week after their preparation, to diminish the risk of degradation of the incorporated antioxidants*."

What do you propose to improve the antioxidant stability of the modified membrane with α-TCP e ALA over storage time, for its future clinical use as HM available commercially? You should comment more deeply the future clinical use of this type of HD membranes.

4- Pag 141- In the last sentence you comment that "… *although without statistical significance, the highest AA level was found for PSf - ALA membranes, which could reflect the synergistic effect of ALA with other antioxidants*". Do you want to comment on this in a future approach perspective?

5- Pag 167-170- Based on your experience and on the preliminary results achieved in the development of molecular imprinting polymers specific for β-cresol and since the direct incorporation of MIP particles of specific size

into PSf membranes does not compromise its structure, can you propose, in short, a research design for the future work in this important area. In view of the fact that a review publication is currently being written.

6- A final question:

Based on the different preliminary results obtained in the studies you carried out, and if you had the opportunity to have funding for one single research project, what would you propose to do in this research area?

Perhaps Michaela could comment on this.

Coimbra, 14th of August 2020

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