



Reviewer's Report on Dissertation Thesis

Student: Mgr. Alice Šimonová

Thesis title: Capillary electrophoresis for liposome analysis and drug interaction studies

The thesis focuses on investigating the possibilities for analyzing and characterizing liposomes using capillary electrophoresis. The introduction provides an overview of liposomes, their characteristics and preparation, as well as their analysis through capillary electrophoresis and capillary coating procedures. The experimental section explores the analysis of phosphatidyl-based liposomes, examines various capillary coating procedures to prevent interactions between liposomes and the inner surface of the capillary, and studies the interactions between liposomes and model pharmaceutically relevant compounds.

The work presented in this thesis is based on three publications (one submitted) in journals with an impact factor, with A. Šimonová as the first author of all of them. Additionally, the candidate is a co-author of 10 other publications. These outputs have already been reviewed by professionals in the field, making them well-suited to serve as foundational elements of the thesis.

The subject of this thesis is highly important, as liposomes are used in the pharmaceutical industry, particularly as drug carriers in vaccines. I personally appreciate the efforts described in this thesis, as the characterization of 'larger objects,' such as liposomes, using capillary electrophoresis is a challenging task. Additionally, I also value the comprehensive work on capillary coating procedures presented in the thesis.

Formally, the thesis is written in English and is easy to read. However, I would have expected a bit more information regarding the last part ('API') directly in the text. For example, on p. 25 ('tissue extract liposomes') and p. 51, I initially thought there was a preparation setup that had not been described until I read the submitted manuscript in the appendix. Similarly, section 3.4.5 (p. 54) describes the effect of pH in a brief paragraph without any examples or explanations, which seemed unusual to me. Again, this information can be found in the appendix. Including some details about liposome

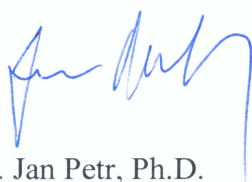
stability, such as pH in normal vs. cancerous tissue, could also be beneficial. Additionally, there are some typos in Figures 20, 22, and 23. However, these points do not significantly affect the overall high quality of the thesis.

I have following questions/remarks for the discussion:

- Did you measure the zeta potential of the liposomes (and its distribution), ideally in the BGEs studied (e.g., p. 28)? Or do you have any information on the zeta potential of your liposomes from previous papers?
- A paper by Rodriguez and Armstrong came to mind when I saw Fig. 13B. They used CTAB-based electrolytes for online preconcentration (aggregation) of bacterial zones in CE (Anal. Chem. 78, 2006, 4759). Do you think it would be possible to aggregate liposomes using CTAB as well? Could the liposome zone be destabilized by increasing the ionic strength?
- Could you explain what is happening with the zones in the capillary for Fig. 23 (p. 47)? Do you have any idea what the peak at 3–4 minutes represents (the blue line; 20 kV, probably 50 s)?

In summary, the candidate has conducted a substantial amount of insightful research and obtained new, original results. The thesis work has been carried out at a high scientific level. Alice Šimonová has demonstrated significant capability as a scientist, and I therefore strongly support the thesis for final defense. In my opinion, the candidate fully deserves to be awarded the PhD degree.

Olomouc, 16/09/2024



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