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Referee's Report on the Ph.D. Thesis

**Development and Evaluation of a Multi-Modal Hyaluronic Acid
Hydrogel for Anti-Inflammatory Drug Delivery for Multiple
Sclerosis Therapy**

by Tutut Ummul Habibah, M.Sc.

submitted to the Faculty of Technology of the Tomáš Baťa University in Zlín

In the past decades, the need for drug delivery systems allowing for continuous release of the drug, thus maintaining its concentration at the site of action at therapeutic levels has drawn the attention of the research community of pharmaceutical chemists. Tutut Ummul Habibah's dissertation is contributing to the field of controlled release systems, focusing on injectable hydrogels based on chemically crosslinked polysaccharides (hyaluronic acid, HA and chondroitine sulfate, CS), which she uses for encapsulation of anti-inflammatory drugs (antibiotic minocycline and oligopeptide Synthetic Preimplantation Factor). The cationic drugs are encapsulated in the gel matrix by electrostatic interaction with negatively charged polysaccharide networks. The obtained results presented in the thesis have been published in two papers in respected journals with a high IF (Carbohydrate Polymers, Carbohydrate Polymer Technologies and Applications). In both papers, Tutut Ummul Habibah is the first author.

The thesis consist of five parts: (1) Introduction, providing information about the current state of art in multiple sclerosis treatment, hydrogels and PECs and their use as drug delivery systems; (2) Aims of the Thesis; (3) Experimental Sections, summarizing the preparation of the drug-loaded hydrogels and their characterization (gelation time, swelling ratio, viscoelastic properties) and about the drug release experiments; (4) Results and Discussion, providing the data from the characterization of drug-loaded hydrogels, release experiments and biological activity test and the interpretation of the data and (5) Conclusion.

The fact that the thesis is concise and written in good and clear English, and the results presented there already passed through the strict review process in two recognized journals does not give me much opportunity for criticism, perhaps with the exception of these few minor comments:

(1) The discussion of PECs formation and of ion encapsulation (1.2.7, p. 26) in PECs is oversimplified. The PECs formation is not driven by electrostatic attraction between polyanions and polycations but by entropic gain from small counterions released upon the complex formation. The same holds for ion encapsulation which means that monovalent ions do not show any affinity to PECs as long as only electrostatic complexation is considered (replacement of one monovalent counterion for another yields no entropic gain.) The ionization state of encapsulated ions in the PEC can be very different from that in the bulk

solution because of charge regulation, and for large polyions such as peptides not only the net charge but also the charge distribution (charge patches) decide whether the ion is encapsulated in the PEC or not.

(2) The way the author presents numerical values is sometimes confusing. For instance, on p. 45, the polymer density $1,299,000 \text{ g m}^{-3}$ would mean, as all these zeros count as significant digits, that the density is known with the accuracy of $\pm 0.5 \mu\text{g cm}^{-3}$ which would be truly remarkable. Another example, p. 44, $M_n 209,352 \text{ g mol}^{-1}$, are all digits significant or is it just an unrounded result of a calculation? (There are more instances of this way of writing numbers in the text.)

(3) In eq 3.7 (p. 44), M_n does not stand for the molar mass of the monomer but for the molar mass of the polymer in the absence of the crosslinking agent. If the author wants to refer to the no-crosslinked polymer as to the monomer, it should be mentioned in the text to avoid any confusion.

(4) A few typos: Tables 3.1, 3.2, 3.3 and 3.4 (pp. 39, 50, 51): “ μ ” are replaced by squares in the text. Several references do not have correct links to the papers, for instance [69]: [https://doi.org/https://doi.org/10.1016/0378-5173\(83\)90064-9](https://doi.org/https://doi.org/10.1016/0378-5173(83)90064-9) while the correct link is [https://doi.org/10.1016/0378-5173\(83\)90064-9](https://doi.org/10.1016/0378-5173(83)90064-9).

For the discussion during the defense, I have several questions for the author:

(1) Considering the data on p. 45, the length of the monosaccharide unit, $l=0.48 \text{ nm}$, and the molar masses of disaccharide repeating units, M_r , 400 g mol^{-1} for HA and 600 g mol^{-1} for CS, the contour length of a strand connecting two crosslinks with the molar mass M_c would be, $L = 2M_c/M_r$, which however yields values which are comparable or even lower than correlation lengths ξ (p. 68) calculated from eq. 3.18. Could you comment on that?

(2) Crosslinking densities of about 10 mol m^{-3} (Table 4.3, p. 67) correspond to the distance between crosslinks of ca. 5 nm . How does this value correspond to ξ values (Table 4.4, p. 68)?

(3) The Korsmeyer-Peppas model (eq. 3.6, p. 43) which diverges for $t \rightarrow \infty$, should be used for fitting the kinetic data at the initial stages of the release, that is, for $m(t)/m_\infty \ll 1$, but it is not the case in your evaluation. Could you comment on that? Wouldn't a model assuming that at $t \rightarrow \infty$, $m(t)/m_\infty \rightarrow 1$ be more suitable for your datasets?

I have to stress that above-mentioned errors do not decrease the scientific value of the submitted thesis. My general opinion is that the work of Tutut Ummul Habibah, M. Sc. represents a significant contribution in the field of drug delivery systems. In summary, I fully recommend accepting the submitted thesis for the defense.

Prague, July 30th, 2025

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