

Dear Dr. Nikolić and Dr. Nedić,

We would like to submit our manuscript entitled “Maltose-mediated long-term stabilization of freeze- and spray- dried forms of bovine and porcine hemoglobin” by Ivana Drvenica, Ana Stančić, Ana Kalušević, Smilja Marković, Jelena Dragišić Maksimović, Viktor Nedović, Branko Bugarski and Vesna Ilić, to be published as an original scientific paper in the journal “*Journal of Serbian Chemical Society*” within category *Biochemistry and Biotechnology*.

Slaughterhouse blood represents a valuable source of hemoglobin, which can be used in production of heme-iron based supplements for prevention/treatment of iron-deficiency anemia. However, a cost effective and feasible process on industrial scale for hemoglobin conversion into long-term stable solid form has not been developed so far. In the present study, we assessed the impact of low-priced sugar maltose on the stability of isolated bovine and porcine hemoglobin, transformed to solid state by spray- and freeze-drying.

Differential scanning calorimetry showed that maltose addition in hemoglobin solution shifted starting point of thermally induced denaturation and melting point of dried hemoglobin forms toward higher values, confirming its protective effect. After being stored for two years at room temperature protected from moisture, reconstituted solid formulations of bovine and porcine hemoglobin with maltose retained color and physico-chemical characteristics same as the starting hemoglobin solution and prevented formation of biologically inactive methemoglobin, as verified by dynamic light scattering and UV-VIS spectroscopy. Comparable quality of solid-state freeze- and spray-dried formulations with maltose with hemoglobin solution stored at -20°C opens the possibility to avoid low temperature ($\leq -20^{\circ}$) storage which is dependent of bulky cooling equipment and impractical. Although encouraging, these results on maltose use as stabilizing additive indicate the need for more specific optimization of drying processes parameters themselves, with an aim of production of long-term stable solid-state formulations of hemoglobin.

The manuscript contains 15 typewritten pages with 7 multiple-panel figures and 1 table. One figure S1 is provided as Supplementary material.

All authors of the manuscript agree to its submission and that the corresponding author has the authority to act on their behalf in all matters pertaining to the publication of the manuscript. We disclose any financial and personal relationships with other people or organizations that could inappropriately influence our work. The manuscript has not been previously published elsewhere in any language and is not currently under consideration by any other publication.

List of potential reviewers:

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Sincerely yours,

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