**REVIEW**

on the dissertation for the degree of "doctor" at the Institute of Chemical Engineering at the Bulgarian Academy of Sciences

**Author of the dissertation:** Master of Science, Eng. Apostol Georgiev Apostolov

**Topic of the dissertation:** "Innovative methods for separation and purification of bioactive substances"

**Direction:** 4.2. Chemical Sciences

**Scientific specialty:** processes and apparatus in chemical and biochemical technology

**Reviewer:** Prof. Dr. Venko Nikolaev Beshkov

## 1. Biographical data of the candidate

Mr. Apostol Apostolov was born in 1995. He received his Bachelor's degree in Chemical Engineering and Technology in 2018 at the Russian University of Chemical Technology "D.I. Mendelev" in Moscow, RF. He received his Master's degree at the same university in 2020 in the specialty "Organization and Management of Knowledge-Intensive Production". During the period 2019/20 he worked as an intern at the Institute of General and Inorganic Chemistry at the Russian Academy of Sciences. Since 2020 he has been a full-time doctoral student at the IIH-BAS. He has participated in 5 research projects (four funded by the National Research Foundation-Bulgaria and one at the Center for Competence HITMOBIL) as an executor. He was awarded the Ivan Evstratiev Geshov Award for Young Scientists of the Bulgarian Academy of Sciences in 2025.

## 2. Relevance of the problem being developed

The interest in this type of research is determined by the development of green technologies for the production of biologically active substances with applications in pharmacy, everyday life and organic synthesis. The topic of the dissertation concerns extraction methods for extracting and concentrating target substances at low energy consumption. The possibilities for using specific solvents (deep eutectic solvents, DES), membrane separation processes (liquid membranes, nanofiltration, reverse osmosis) have been studied.

207 literary sources are cited in the dissertation work, which are distributed in time as follows: before 1990 10; for the period 1991-2000 – 7; 2001-2020 – 106; after 2020 – **84.** This shows that the interest in this research is great and that the topic is relevant, and the author is familiar with the latest achievements in the field.

**3. Does the dissertationist know the state of the problem and creatively evaluate the literary material?**

In the literature review, the author focuses on the development of extraction processes over the years and on the importance of the processes studied in the dissertation work.. The goal and objectives of the dissertation work are derived from the literature review and are very well formulated. The overall impression is that the dissertationist has a good command of the literary material and handles it freely.

**4. Brief analytical characterization of the nature and assessment of the reliability of the material on which the contributions of the dissertation work are built**

The author presents three different methods for extraction in "liquid/liquid" systems, combined with membrane methods for extracting biologically active components from liquid media. First of all, this is extraction with "deep eutectic solvents" (DES). The latter contain combinations of organically soluble components for the selective extraction of target components with the starting solutions.

The properties of five active components of DES have been synthesized and studied: menthol in combination with four alkyl amines (dioctyl-, trioctyl-, trihexyl- and tridodecyl-amines) and menthol with salicylic acid.

Another group of membrane methods (nanofiltration, reverse osmosis and diafiltration). They use the difference in molecular mass and the corresponding sizes of the molecules of the dissolved substances. In third place comes pertraction (a combination of extraction and liquid membranes, in which extraction and re-extraction occur simultaneously). This method is not new, but in the dissertation it is demonstrated and applied to the separation of stereoisomers (maleic and fumaric acids), based on the different solubility of cis- and trans-isomers in aqueous and organic media.

Modern research methods have been selected. The analyses are based on high-performance liquid chromatography, thermogravimetric analysis, viscometry (where necessary) and Fourier transform infrared spectroscopy to characterize the obtained DES.

**5. What are the scientific and applied scientific contributions of the dissertation?**

The dissertation is mainly practical in nature, and the contributions in it are applied scientific. More generally, they are as follows.

• The extraction of L-lactic acid with DER containing menthol amines was studied, with the best extraction being found in solvents with dioctyl and trihexyl amines.

• A pronounced selectivity of menthol salicylic acid towards fumaric acid (trans-isomer) compared to maleic acid (cis-isomer) was found.

• When using pertraction, the effect of separating the two stereoisomers is manifested in an acidic medium, while in an alkaline one it is suppressed. This is to be expected, since the anions of the two acids have the same affinity for water, unlike their behavior in an acidic medium. The contributions so far are of significant scientific importance.

• The possibilities for membrane extraction of components of wine of the “Mavrud” variety using nanofiltration, in combination with reverse osmosis, as well as diafiltration, were investigated. The properties of two types of nanofiltration membranes and two types of reverse osmosis membranes were investigated. Different flow organization modes were also investigated. As might be expected, substances with a lower molecular mass (ethanol and acetic acid) pass through the membranes. This research and its contributions are clearly applicable, although in the more distant future.

6. Can it be assessed to what extent the dissertation work and contributions represent the personal work of the dissertation candidate?

The presentation of the texts of the dissertation and the abstract show that the development of the dissertation is the work of the doctoral student.

**7. Critical notes**

There are the following remarks on the writing and formatting of the texts in the dissertation.

• On page 19, it talks about high-voltage electrical pulses, but in fact it should be of high intensity (kV/cm).

• On page 20, it talks about "mass transfer as a thermodynamic transfer (?) and its rate depends on temperature. What does this mean?

• What does "donor/acceptor" of a hydrogen bond mean?

• On page 26, it talks about "amplification" of the process, instead of acceleration.

• It would be more appropriate to formulate the goals and objectives of the dissertation at the end of Chapter 1, and not separately as Chapter 2.

• It is not correct to write "under acidic" or "alkaline conditions", but in an acidic or alkaline environment.

**7. Assessment of publications in the dissertation work**

The dissertation work is based on three scientific publications in journals with Q2 (two) and one with a journal with Q4. In the abstract, the bibliography of publication 3 is not fully listed and it is not clear whether the dissertation candidate is its author. One citation of publication 2 from the abstract has already been noted.

The results of the research have been reported at 3 international scientific forums, and another five - at conferences in Bulgaria.

The scientific production on which the dissertation is based, as well as the credits collected during the doctoral period, fully meet the requirements for the award of the educational and scientific degree "doctor" according to the regulations of the IChE-BAS.

**8. Is the abstract prepared in accordance with the requirements, does it correctly reflect the main points and main contributions of the dissertation work?**

The abstract correctly reflects the content of the dissertation work.

**9. Conclusion**

The above allows me to confidently recommend to the esteemed jury to award the scientific degree "doctor" to the master. Apostol Georgiev Apostolov.

Sofia, December 19, 2025

REVIEWER:

Prof. Dr. Venko Nikolaev Beshkov