# REVIEW

on Dissertation Defense

**Innovative methods for the separation and purification of bioactive substances**
*(Title)*

for acquiring the educational and scientific degree "doctor"

in the scientific specialty **Processes and apparatus in chemical and biochemical technology**

with a candidate: **Apostol Georgiev Apostolov**
*(name, scientific degree, academic position)*

Reviewer: **Assoc. Prof. Dr. Dimitar Tsvetkov Peshev**
*(name, scientific degree, academic position)*

## 1. Biographical Data and Scientific Interests of the Doctoral Student

Apostol Georgiev Apostolov was born on April 7, 1995. He holds a master's degree with honors in digital technologies and chemical engineering from the Russian University of Chemical Technology 'D.I. Mendeleev', where he also completed his bachelor's degree in chemical engineering and technologies. Before his doctoral studies at IChE-BAS, he gained research experience in analytical chemistry and chemical engineering during an internship at the Institute of General and Inorganic Chemistry – Russian Academy of Sciences, from February 2019 to July 2020. Since 2021, he has been a regular doctoral student at IChE-BAS and also serves as the safety and health officer. His scientific interests include process analysis and modeling, equipment design, and high-performance liquid chromatography. He has contributed to four national scientific projects and has 18 publications and 10 citations, some of which are outside the scope of his dissertation.

## 2. Relevance of the Dissertation Topic.

The dissertation focuses on designing and testing sustainable and 'green' technologies for separating and purifying bioactive substances. The goal is to improve the efficiency, selectivity, and environmental sustainability of these processes, aligning with modern requirements for producing natural resource-based products. The work explores three main methods: liquid-liquid extraction using deep eutectic solvents (DES) as an eco-friendly alternative to organic solvents; separation with liquid membranes (pertraction); and separation under 'mild' conditions using solid membranes (nanofiltration and reverse osmosis) as an alternative to thermal methods. The use of DES and nanofiltration for extracting and purifying bioactive substances has garnered significant interest in the chemical and biotechnological industries due to their high selectivity, low energy consumption,

and preservation of target component structure and activity. The topic and results align with the National Strategy for Research Development and European Commission challenges

**3. Review of the Dissertation and Analysis of Results.**

The dissertation begins with a comprehensive overview of both conventional and alternative/intensive technologies for separating and purifying biologically active substances, including solid-liquid extraction, liquid-liquid extraction, membrane separation processes, chromatographic methods, and instrumental chemical analysis. The analysis effectively justifies the selection of intensive and 'green' technologies, such as DES-assisted liquid-liquid extraction and nanofiltration, for the experimental research. Pertraction with organic solvents is not considered a 'green technology', but the doctoral student's hypothesis that future research could involve DES-based membrane phases justifies its inclusion.

New hydrophobic DES systems based on menthol have been successfully synthesized and characterized. Menthol-amine DES, including dioctyl amine (DOA) and trihexyl amine (TXA), were shown to be highly effective for L-lactic acid extraction. The stability of M-DOA was studied over multiple extraction and re-extraction cycles, showing effectiveness for up to four cycles before partial capacity filling. The reviewer notes that it is not commented whether this result is satisfactory for practical application or how residual DES is utilized. Menthol-salicylic acid DES exhibited clear selectivity for the *trans*-isomer (fumaric acid) of butenedioic acid, suggesting its potential as a selective extractant for geometric isomers.

Pertraction was demonstrated to effectively separate butenedioic acid isomers, achieving optimal selectivity for fumaric acid under acidic conditions (pH 1.8) using a 1-decanol membrane. Alkaline conditions significantly inhibited mass transfer due to complete acid dissociation.

The potential of nanofiltration and reverse osmosis was shown for fractionating red wine. Commercial polymer membranes can effectively separate wine components from the solvent, including ethanol, at ambient temperature. A combined dia-nanofiltration/reverse osmosis scheme achieved 90-100% retention of key components and effective reduction of ethanol concentration in wine. All experimental results are original, obtained using relevant methods and equipment, and presented correctly.

**4. Main Scientific and Applied Contributions**.

The dissertation's scientific and applied contributions include enriching knowledge on the synthesis and fundamental characteristics of new green hydrophobic deep eutectic solvents (DES) and clarifying their selective mechanisms for liquid-liquid extraction. It also contributes to the practical application and optimization of nanofiltration for industrially significant separations, particularly in the food and biotechnological sectors.

The results on selective extraction with menthol-salicylic acid DES enhance understanding of DES properties and potential applications. The clear selectivity of menthol-salicylic acid DES for the trans-isomer (fumaric acid) of butenedioic acid is attributed to different distribution mechanisms related to molecular geometry, hydrogen bonding, and hydrophobic interactions with the organic phase.

The reported results on partial dealcoholization of 'Mavrud' red wine through filtration with nanofiltration and reverse osmosis polymer membranes have significant scientific and applied character. A two-stage process using commercial 'Dia-NF/RO' membranes significantly reduces alcohol content with minimal loss of organic acids and sugars, providing practical guidelines for industrial red wine dealcoholization.

The doctoral student's publications indicate effective participation in research teams from three BAS institutes, utilizing established topics, experimental installations, methodologies, and analytical instruments. While not creating new scientific themes or theories, the scope and quality of the research exceed the requirements for obtaining a 'Doctor' degree.

**5. Description and Evaluation of Presented Materials**

The doctoral student submitted three scientific publications in impact factor journals, which count towards indicator 7 of group G under the Regulations for the Development of Academic Staff in Bulgaria, Area 4, PN 4.2. In one publication, the doctoral student is the first author, achieving 52 points in group G, exceeding the minimum requirement of 30. This demonstrates that the publication activity surpasses national requirements and those of the IChE Regulations for scientific degrees. All scientific publications are relevant to the dissertation topic.

**6. Impact of the Candidate's Scientific Publications.**

The Scopus database shows only one independent citation for the candidate's works included in the dissertation. Additionally, there are 12 other citations for 12 of the doctoral

student's publications not included in the dissertation.

**7. Critical Remarks and Recommendations.**

The doctoral student's publications are in reputable, indexed scientific journals and meet relevant standards. However, the dissertation's preparation showed carelessness, leading to technical errors and inaccuracies. The literary sources do not cite the candidate's own publications that form the basis of the dissertation.

**8. Reviewer's Personal Impressions.**

I have no personal impressions of the doctoral student.

**CONCLUSION**

I believe that the dissertation 'Innovative methods for the separation and purification of bioactive substances' by Apostol Georgiev Apostolov meets the requirements for scientific-applied research for the 'Doctor' degree, as per Bulgarian law and relevant regulations. The dissertation and the doctoral student's personal contribution are positively evaluated.
I recommend that the Bulgarian Academy of Sciences awards Apostol Georgiev Apostolov the educational and scientific degree of 'Doctor'.

Date: Reviewer:

22.12.2025 / Assoc. Prof. Eng. Dimitar Peshev /