Opinion

Regarding the PhD thesis titled "Innovative methods for separation and purification of bioactive substances."

For the attainment of the educational and scientific degree of "Doctor" in the professional field 4.2 "Chemical Sciences," specializing in Processes and Apparatus in Chemical and Biochemical Technology, candidate Apostol Georgiev Apostolov.

Opinion author: Prof. Dr. Milen Georgiev Bogdanov, Sofia University St. Kliment Ohridski.

1. **Brief biographical information and overview of the doctoral candidate's scientific interests and activities.**

Apostol Georgiev Apostolov is a researcher specializing in chemical engineering. He graduated in 2018 from the Russian University of Chemical Technology "D.I. Mendeleev" (RKTU) with degrees in chemical engineering, technology, logistics, and economics. In 2020, he earned a master's in industry and digital economy from the same university, both after defending his theses. Since 2021, he has been a doctoral candidate at the Institute of Chemical Engineering at the Bulgarian Academy of Sciences (IICH-BAS). His career includes internships in the "Analytical Chemistry and Separation Methods" laboratory at the Institute of General and Inorganic Chemistry - Russian Academy of Sciences (IONKh-RAS) and a position as an assistant at the Institute of Chemical Engineering - BAS from 2020 onward. During his academic and research activities, PhD student Apostolov gained extensive knowledge in chemical technologies, process management and quality, and experience with high-performance liquid chromatography. He has participated in six scientific projects and is a co-author of three publications in peer-reviewed international journals related to his dissertation topic.

2. **Significance of the problem addressed in the dissertation.**

The dissertation titled "Innovative methods for separation and purification of bioactive substances" tackles a key issue: the efficient, selective, and eco-friendly extraction of organic molecules from different matrices. The novel experimental extraction techniques offer a viable alternative to traditional methods like liquid-liquid extraction using organic solvents, which come with several drawbacks, higher risks, and environmental concerns.

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

The dissertation encompasses 110 pages and includes 37 figures and 19 tables to illustrate and summarize the content. The bibliography contains 207 scholarly sources. The writing

style is academic, presenting information in a clear, consistent, and comprehensive manner. Adequate figures and diagrams of the equipment are incorporated, clearly demonstrating the involved processes. A thorough account of the experimental conditions and a logical discussion of the results underscore the candidate's rigorous preparation and potential as a young scientist in the fields of chemical sciences and technologies..

4. **Primary Scientific and Applied Scientific Contributions**.

Apostol Apostolov has thoroughly reviewed existing literature on extraction methods and their applications. He has demonstrated strong practical skills, leading to research summarized as follows:

• Conducted a synthesis and investigation of density, viscosity, thermal stability, and intermolecular bond formation (via FTIR) of a series of deep eutectic solvents (DES) based on menthol combined with four amines and menthol combined with salicylic acid, across various molar ratios.

• The resulting DESs were employed as an organic phase for the liquid-liquid extraction of organic acids—lactic acid and both isomers of butanedioic acid—and their efficiency was analyzed in relation to composition, alongside the possibilities for regeneration and reuse.

• Developed a method for separating the two isomers of butanedioic acid through pertraction under varying pH conditions, establishing selectivity towards one isomer.

• Investigated the feasibility of extracting organic acids, sugars, and alcohols from “Mavrud” wine with the primary aim of reducing acetic acid and ethanol levels via nanofiltration and reverse osmosis, and evaluated the effect of different membrane types.

5. **Description and assessment of the submitted materials:**

The research findings related to the dissertation topic have been published in three scientific journals, as documented by the candidate (Chemistry, Q2; Applied Sciences, Q2; Theoretical Foundations of Chemical Engineering). This achievement accrues 52 points, surpassing the minimum requirement of 30 points set by the IIH Regulations for the acquisition of scientific degrees..

6. **Reflection of the candidate's scientific publications in both Bulgarian and international literature..**

A scientific publication on this topic has been cited by international authors, demonstrating the research significance..

7. Critical comments and suggestions regarding the candidate's scientific publications.

The dissertation is well structured, offering detailed information on the candidate's experiments and results. Although there are some technical errors in certain sections, they do not hinder the overall clarity or effective communication of the content. I have a few questions for the candidate:

- What was the reason for choosing lactic acid and butanedioic acid (cis- and trans-) as research subjects?
- Why were menthol/salicylic acid and menthol/amine selected as the components of the DES?
- Have experiments been conducted on the extraction of lactic acid or butanedioic acid at temperatures higher than ambient, and has the impact of viscosity on the extraction process been examined?

CONCLUSIONS

This dissertation provides a thorough analysis of a current issue, including an in-depth literature review, rigorous research, and careful organization of the results. **I highly recommend Apostol Apostolov for the award of the 'Doctor' degree.**

Date

Reviewer:
/Prof. Dr. Milen Bogdanov /