SCIEN|TIFIC APPRAISAL

by Prof. Dr. Boris L. Shivachev,

member of the Scientific Jury appointed by order 115RD-09/ 27.04.2021 of the Director

of the Institute of Mineralogy and Crystallography "Acad. Ivan Kostov"

Address: Acad. Georgi Bonchev Str., Bl. 107, 1113 Sofia

of the thesis for awarding the educational and scientific degree 'doctor'

in the **field of higher education**: Natural Sciences, Mathematics and Informatics,

Professional field: “4.4. Earth Sciences ",

Doctoral program: "Mineralogy and crystallography".

Author: RUSI IVANOV RUSEV, full-time PhD student at the Institute of Mineralogy and Crystallography "Academician Ivan Kostov" – BAS

Title: "SYNTHESIS, STRUCTURAL CHARACTERISTICS AND ANTIMICROBIAL ACTIVITY OF QUATERNARY AMMONIUM COMPOUNDS"

Scientific adviser: Prof. Dr. Boris L. Shivachev, IMC – BAS

The PhD Thesis (dissertation) developed by Rusi Rusev is presented on 157 pages with 52 figures, 8 tables and 41 schemes of the compounds included in the text (not counting the additional figures, schemes, tables, etc. presented in the four appendices with a total volume of 79 pages). Two hundred fifty-five (255) literature references are cited. The dissertation structure is traditional and contains the following chapters: Introduction, Actual state-of-the-art review, which ends with the Thesis Aim and the Tasks to be performed, Materials and methods, Results and discussion, Conclusion and contributions, cited References and Appendices.

The subject of the dissertation is in the trending research field of new antibacterial agents - namely quaternary ammonium salts (QAS). The investigated materials are new, they are obtained (synthesized) on the basis of a suitably selected reaction mechanism and a scheme corresponding to their preparation in appropriate quantities and purity in view of the planned subsequent studies of their potential antibacterial properties. In this sense, the research subject is promising, with potential practical orientation and can serve as a basis for future research.

The aim of the present dissertation is clearly formulated with the end goal being the synthesis of a series of new Quaternary ammonium salts (QAS) by the use of a simple, reproducible and cost-effective synthetic protocol. The characterization of the newly obtained QAS will be performed by the means of spectroscopic, structural X-ray diffraction and thermal techniques, and in a view of the antibacterial application by susceptibility test disk diffusion method (Kirby-Bauer) and broth micro-dilution (MIC and NIC).

The **literature state-of-the-art review** is presented on 20 pages, and is divided into two subsections. Without making obvious conclusions in an explicit form, it is the basis for defining the aim and the objectives (tasks) of the dissertation.

The **Materials and Methods** section covers 23 pages including: the description of the synthetic protocols for the preparation of the new QAS, NMR data from their characterization, brief description of the equipment and the conditions under which the spectral (IR, UV, NMR and fluorescence), X-ray diffraction (powder and single crystal X-ray diffraction analysis) and thermal (DSC) analyzes were carried out.

The **Results and Discussion** section expands and systematizes the data for the newly obtained QAS. It covers a volume of 90 pages from the thesis and is divided into several subsections: Synthesis and purification; Determination of purity and molecular structure (in solution); Determination of the crystal structure of QAS (solid state); DSC thermal studies; Spectroscopic characterization; Antibacterial studies and structure-activity-relationship (SAR) regarding the antibacterial activity. The preparation of 29 new quaternary ammonium compounds was shown, for 25 of them crystals were grown and the crystal structures were solved, the thermal properties (stability, solvent release, melting and destruction) were studied. The obtained substances were tested for antibacterial effect on *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherihia coli, Staphylococcus aureus and Bacillus Subtilis* - by the Kirby-Bauer susceptibility disk diffusion method and by broth micro-dilution. The Results and Discussion part ends with the analysis of the relationship "Structure-antibacterial activity" for the synthesized QASs. From the SAR analysis a specific sequence of steps (protocol) for future design of new antibacterial CASs is derived and is clearly systematized in a schematic diagram.

The presented **Conclusions** and the designated **Contributions** summarize the previous sections and show that the PhD student Rusi Rusev has successfully managed all of the objectives set in the begging of the dissertation and achieved the aim of the thesis.

I have no critical comments regarding the dissertation. My “work” supervising MSc. Rusi Rusev at the IMC, related to the dissertation and to other scientific tasks not related with the current dissertation preparation, was a pleasure. I consider Rusi Rusev to be an established specialist in the field of structural research.

In conclusion, I believe that the PhD dissertation of Rusi Rusev deals with an interesting and up-to-the-minute problem related to the synthesis of new QAS and the study of their physicochemical and antibacterial properties. The conducted research is at a high experimental level, performed with the use of modern techniques and equipment, and the results are critically and thoroughly discussed. The presented dissertation manuscript and the volume of the performed scientific work fully correspond in volume, methodical level, number of cited references and number of published manuscripts by R. Rusev in scientific journals to the Requirements specified in the Law for development of the academic staff, Regulations for application of the law for development of the academic staff and the criteria of the Institute of Mineralogy. and crystallography "Acad. Ivan Kostov" - BAS.

Based on the above, I give a positive assessment for and propose to the members of the Scientific Jury to award **Rusi Ivanov Rusev** the educational and scientific degree "Doctor" in the professional field "4.4. Earth Sciences”, doctoral program: “Mineralogy and Crystallography”.

Boris Lyubomirov Shivachev

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June 12 2021 prof. Boris Shivachev