

REVIEW

by Assoc. Prof. Dr. Luiza Dimova Terziyska,
Institute of Mineralogy and Crystallography, "Acad. IVAN KOSTOV" BAS
member of the Scientific Jury, appointed by order № 115 ПД-09-180/27.04.2021

on PhD thesis for the award of the educational and scientific degree "doctor", field of higher education 4.4 Earth Sciences, PhD program "Mineralogy and Crystallography"

Author: **Rusi Ivanov Rusev**, PhD student at the Institute of Mineralogy and Crystallography BAS

Subject: **"Synthesis, structural characteristics and antimicrobial activity of quaternary ammonium compounds"**

Scientific Advisor: Prof. Dr. Boris Shivachev

1. Relevance of the problem developed in the dissertation in scientific and/or scientific-applied terms.

The dissertation developed by the doctoral student Rusi Ivanov Rusev is focused on class ionic compounds - Quaternary ammonium salts (CAS), widely used as antimicrobial agents. CASs have long been known and used as disinfectants. In the last year, such researches have become relevant because of the pandemic situation. These compounds are suitable in scientific-applied aspect: they are useful in areas such as medicine, where they are studied for antitumor and antimalarial activity, also are used as antifungal antimicrobial agents. CAS are also attractive in industry as catalysts for interphase transfer, reagents in organic synthesis, they are ideal ionic liquids with different applications, as well as softeners, cationic surfactants and others.

This work is a modern study that presents a developed protocol for fast and easy methodology for synthesis of CAS, supported by 29 obtained new compounds, almost all of which are presented with their structures and properties. The elucidation of the mechanisms of the synthetic process, as well as the characterization of the new CASs is an actual scientific contribution in the respective field. The new CASs presented in the dissertation can be further studied in various scientific fields such as medicine, biology and those of industrial significance.

2. Is the candidate Rusi Ivanov Rusev familiar with the state of the problem and does he creatively evaluate the literary material?

A short and clear structural characteristics of CAS presented in the introduction reasonably pointed why the improvement of the synthesis method is necessary as well as CASs antibacterial usage is discussed. The purpose of the dissertation is well grounded: to synthesize a series of CAS by applying an easy, reproducible and cost-effective synthetic protocol. To achieve this goal the tasks are comprehensively and logical formulated.

The PhD student is well acquainted with the state of the scientific matter he works on and the citing references show that clearly. In the section "Literature Review" the methods for obtaining CAS are considered, the author explains in depth the peculiarities of the atomic

structure of amines; a similar addition are the explanations of CASs ionic structures; the mechanisms of synthesis are clarified; the scientific decisions of presented synthetic protocol are well founded. The literature comprehensively covers the various applications of CAS widely used in medicine, biological research, industry etc.

Guidelines of the Introduction show motivated choice of topic and scientific decisions for the work planned. It is clear that Rusi Rusev knows well the state and development of the scientific issues chosen for his dissertation, as well as prospects for future research.

3. Can the chosen research methodology answer the set goals and objectives of the dissertation?

The choice of the scientific problem and the detailed literature reference substantiate the purpose of the dissertation and seven tasks for the implementation of this work are marked. The tasks of synthesis and optimization of the synthetic protocol are related to methods for identification and characterization of the obtained substances such as powder X-ray diffraction and single crystal X-ray diffraction analyzes, nuclear magnetic resonance spectroscopy, differential scanning calorimetry. UV/VIS, infrared (IR-FT) and fluorescence spectroscopy were used for the spectral characteristics of CAS. In the stage of studying the antibacterial effect of the obtained CAS against two Gram strains-positive and three Gram strains-negative microorganisms, the Kirby-Bauer disk diffusion method was used. Broth micro dilution (BMD) was used to determine the initial and minimum inhibitory concentrations of the active substances.

The selected methods are well described and appropriately used in carrying out the set tasks. A large amount of experimental work has been performed, new crystalline materials have been obtained as raw x-ray data, successfully refined and presented as crystal structures, their purity has been evaluated and absorption, fluorescent and antibacterial properties have been studied.

4. Analytical characteristics of the representativeness and reliability of the material on which the contributions of the dissertation are built.

The dissertation work covers the synthesis protocol, preparation and characterization of the new CAS and is presented in 7 sections using traditional model, namely: Introduction, Literary Review, Materials and Methods, Results and Discussion and Conclusion, Literature, Applications. The dissertation consists of 157 pages and 52 figures, 41 schemes, 8 tables are attached to it, and 255 reference sources are indicated in the section "Literature". The material is presented competently and reasonably, laconic and concise language is used.

The refined new structures have good reliability factors built into software packages of the X-ray diffraction analysis. The selected methods complement the X-ray structural data, the use of NMR is grounded and necessary for the overall characteristic. The results do not contradict the literature data and successfully supplement the knowledge in this direction. The

correct use of the methods, comparison and interpretation of the results allows the proposed dissertation to claim representativeness and reliability of the data contained in it.

5. What are the scientific and / or scientific-applied contributions of the dissertation:

The dissertation contains comprehensive scientifically presented information and has a direct theoretical and scientifically applied contribution to modern research in the field. In this aspect the results of the dissertation can be presented as:

Development of a modified methodology for CAS synthesis applicable for easy preparation of aromatic heterocyclic analogs.

According to the proposed method, 29 not described in the literature CASs were obtained, derivatives of 4-pyrrolidino pyridine, quinoline and 4,4-bipyridine, having a unique structural model: N-heterocycle - oxoethyl bridge - aromatic substituent.

From the 29 synthesized CAS, 25 new ones were crystallographically solved and described. Some of them are deposited in the crystallographic database - Cambridge Structural Database (CSD).

Five of the synthesized compounds show better antibacterial activity against *Staphylococcus aureus* in comparison to commercially used antibacterial agent - Canamycin. The bacteriologically active compounds can be used as prototypes for future research.

In conclusion, an original attempt was made to determine the relationship "Structure-antibacterial activity" of CAS and a schematic diagram was drawn up in the form of a sequence of steps, which can be used to design new antibacterial-active compounds. This scientific approach, which the candidate has skillfully inserted as an idea for future work in the end of his dissertation illustrated one of the interesting possibilities, which interdisciplinary researches provides.

For the development and realization of the dissertation interdisciplinary knowledge is needed, which the candidate has actively acquired during the performance of the tasks.

6. Personal contribution of the candidate

The results and contributions to Rusi Rusev's dissertation are generally his personal work. The role of his supervisor is essential for improving his knowledge in this field, in planning the work and discussing the problems and results. Rusi Rusev acquired a significant amount of knowledge and practical work during the realization of his dissertation. He works freely with the equipment in IMC: X-ray structural analysis - powder and single crystal, UV / VIS, fluorescence and infrared (IR-FT) spectroscopy, differential thermal calorimetry, can interpret the relevant data and NMR data. The candidate has developed systematic skills in organic synthesis and successfully implements the tasks set in this direction, offering a large volume of newly synthesized CAS. Rusi Rusev made a serious literature survey on the topic, which helped him build his concept of work, as well as logically and motivated to present his work.

My personal impressions of the PhD student are positive. The candidate is actively and persistently working on his dissertation and his personal participation and creative approach are indisputable.

The PhD student has acquired excellent training in the field of CAS synthesis, structural studies, characterization of the antibacterial activity of the obtained compounds and he can perform a similar tasks easily. Important fact is that he received his knowledge in a renowned scientific institute with a long time experience.

7. Estimation of the dissertation publications: number and editions.

The PhD student has 3 published scientific articles in journals with impact factor, two of which are on the topic of the dissertation: Crystals (IF: 2.404, Q2) in 2020 and Bulg. Chem. Comm. (SJR 0.137) and there the PhD student is the first author, which shows his active participation in the research. Rusi Rusev participated in 3 scientific forums during the preparation of the dissertation, as part of the main results were presented at a scientific forum: Seventh National Crystallographic Symposium, with International Participation, 2018, "Synthesis and structure solution of novel quaternary ammonium salts of quinoline and 4,4'-bipyridine".

8. The use of dissertation results in scientific practices.

At least three scientific fields are of potential interest in the results of this work: synthesis and structural characterization of CAS; medical application of CAS; and study of the antibacterial properties of CAS. The dissertation can be of direct interest in the relevant scientific circles and in scientific practice.

9. Critical remarks and motivated recommendations for future use of scientific and / or scientific-applied contributions.

The dissertation is written comprehensively and easy to read. The presented tables, figures and diagrams help for easy assimilation of the discussed information. Permitted linguistic inaccuracies are rare and do not exceed those permissible for this type of presentation.

I have no significant remarks and disagreements in principle with the presented dissertation. The presented material exceeds the requirements for a dissertation, but this could not be a remark. I highly recommend to the doctoral student to continue his research in the direction set by the dissertation, as well as to develop further topics and research.

10. Does the abstract correctly present the main points and scientific contributions of the dissertation?

The abstract objectively and adequately presents the structure and the meaning embedded in the dissertation. The goals, tasks and conclusions correspond without omissions to those formulated in the main work.

Conclusion:

The matter of the thesis is a current topic, as well as undoubted scientific contribution in the chosen field. This work has more than a sufficient volume of research as a dissertation. The

chosen field is promising and is of interest to the scientific community. The PhD student has acquired a set of knowledge and skills and can work in the relevant field. The dissertation fully meets the requirements of the Regulations on the terms and conditions for obtaining scientific degrees and for holding academic positions at the Institute of Mineralogy and Crystallography "Acad. Ivan Kostov " - BAS, as well as satisfies all the requirements of the Law for Development of the Academic Staff in the Republic of Bulgaria (LDASRB) and the Regulations for application of LDASRB and the Regulations of BAS.

I am convinced of my positive assessment and recommend to the esteemed scientific jury to vote for the award of the educational and academic degree "**Doctor**" to Rusi Ivanov Rusev.

Date

14.06.2021

Reviewer:

Заличено съгласно
чл. 2 от ЗЗЛД

/Assoc. Prof./