

TO THE ACADEMIC-TEACHING COUNCIL

Subject: Report on the completed doctoral dissertation of candidate Amjed Karkad

By Decision No. 2026-35/77 dated March 5, 2026, we were appointed members of the Committee for the evaluation of the doctoral dissertation of candidate Amjed Abdullah Mohammed Karkad entitled: "Liposomes and Bio-Polymeric Films for Efficient Controlled Release of Silibinin and *Cotinus coggygria* Scop. Extract".

After reviewing the submitted dissertation and accompanying materials, and after an interview with the candidate, the Committee has prepared the following:

REPORT

1. INTRODUCTION

1.1. Chronology of Approval and Preparation of the Dissertation

Candidate Amjed Karkad enrolled in doctoral academic studies in the study program Chemistry in the academic year 2021/2022 at the Faculty of Technology and Metallurgy, University of Belgrade.

November 28, 2025 – Candidate Amjed Karkad proposed the doctoral dissertation topic entitled:

"Liposomes and Bio-Polymeric Films for Efficient Controlled Release of Silibinin and *Cotinus coggygria* Scop. Extract".

December 25, 2025 – At the session of the Academic-Teaching Council of the Faculty of Technology and Metallurgy, University of Belgrade, Decision No. 2025-35/509 was adopted on appointing the Committee for the evaluation of the suitability of the topic and candidate Amjed Karkad for preparation of the doctoral dissertation under the above-mentioned title.

February 5, 2026 – At the session of the Academic-Teaching Council of the Faculty of Technology and Metallurgy, University of Belgrade, Decision No. 2026-35/20 was adopted, accepting the Report of the Committee for evaluation of the suitability of the dissertation topic and approving the preparation of the doctoral dissertation of Amjed Karkad under the same title.

The following were appointed as mentors:

Prof. Dr. Aleksandar Marinković, Full Professor, University of Belgrade, Faculty of Technology and Metallurgy

Dr. Tamara Erceg, Senior Research Associate, University of Novi Sad, Faculty of Technology Novi Sad

February 26, 2026 – At the session of the Council of Scientific Fields of Natural Sciences of the University of Belgrade, consent was given to the proposed doctoral dissertation topic of Amjed Karkad, Decision No. 02-07 No. 61206-631/2-26.

The appointed mentors were:

Prof. Dr. Aleksandar Marinković, University of Belgrade, Faculty of Technology and Metallurgy

Dr. Tamara Erceg, University of Novi Sad, Faculty of Technology Novi Sad

March 5, 2026 – At the session of the Academic-Teaching Council of the Faculty of Technology and Metallurgy, University of Belgrade, Decision No. 2026-35/77 was adopted, appointing the Committee for the evaluation of the doctoral dissertation of Amjed Karkad.

1.2. Scientific Field of the Dissertation

The research conducted within this doctoral dissertation belongs to the scientific field of Chemical Sciences, for which the Faculty of Technology and Metallurgy, University of Belgrade, is the parent institution.

The mentors, Prof. Dr. Aleksandar Marinković and Dr. Tamara Erceg, based on their long-standing participation in teaching and scientific work, as well as their scientific and professional publications, were fully competent to supervise the preparation of the doctoral dissertation of candidate Amjed Karkad.

1.3. Biographical Data of the Candidate

Amjed Karkad, doctoral student at the Faculty of Technology and Metallurgy, University of Belgrade, was born on January 10, 1985, in Msallatah, Libya. In 2004, he completed secondary school in Rossol Aldachara Msallatah, Libya. He obtained his undergraduate degree in 2009 from the Faculty of Medical Technology, AlFatah University, Tripoli, Libya. He completed his Master's studies in 2020 in Belgrade, Serbia, at Metropolitan University, Faculty of Applied Ecology Futura, in the field of environmental protection, study program Environmental Risk Management, thereby acquiring the title of Graduate Environmental Protection Analyst. In the academic year 2021/2022, Amjed Karkad enrolled in doctoral studies at the Faculty of Technology and Metallurgy, University of Belgrade, Department of Organic Chemistry, under the supervision of mentor Dr. Aleksandar Marinković. He passed all examinations prescribed by the curriculum, as well as the final doctoral examination. The doctoral dissertation was prepared under the mentorship of Prof. Dr. Aleksandar Marinković, Faculty of Technology and Metallurgy, University of Belgrade, and Dr. Tamara Erceg, University of Novi Sad, Faculty of Technology Novi Sad.

The candidate has so far published results of his scientific research in a total of 8 publications, of which:

1 paper in an international journal of exceptional value (M21a)

1 paper in a top international journal (M21)

4 full papers at international conferences (M33)

2 abstracts at international conferences (M34)

His future scientific work will continue in the field of chemical and biochemical technology and its applications. The scientific interests of Amjed Karkad are focused on: extraction and analysis of biologically active compounds from plant sources, encapsulation of such compounds into liposomes, incorporation into innovatively designed biopolymeric films, investigation of biological activity, and potential applications of active compounds in new platforms for future use in the cosmetic and pharmaceutical industries.

2. DESCRIPTION OF THE DISSERTATION

2.1. Contents of the Dissertation

The doctoral dissertation of candidate Amjed Karkad, a doctoral student at the Faculty of Technology and Metallurgy, University of Belgrade, consists of 118 pages (of which 110 pages are numbered), containing: 7 chapters, 38 figures (+15 figures in Supplemental Material), 4 tables (+1 table in Supplemental Material), and 184 references.

The dissertation includes the following sections: Introduction, Aims of Investigation, Experimental Part, Results and Discussion, Conclusions, References, and Supplemental Material.

At the beginning of the dissertation, abstracts are provided in both English and Serbian. In terms of form and content, the submitted dissertation fulfills all standards prescribed by the University of Belgrade for doctoral dissertations.

2.2. Brief Overview of Individual Chapters

In the Introduction, an overview of the biological potential of polyphenols is presented, with special emphasis on silibinin and extracts of the plant *Cotinus coggygria* Scop. (smoke tree). Polyphenols are thoroughly explained as secondary plant metabolites, while the antioxidant, anti-inflammatory, and dermatoprotective properties of smoke tree extracts and silibinin are highlighted. Given the key challenges of low bioavailability and chemical instability of these natural compounds, encapsulation in liposomes was proposed as an efficient strategy to protect them from degradation and enable controlled release. The processes of encapsulation of plant-derived bioactive compounds, as well as post-encapsulation treatments, are described in detail. Lipid-based liposomal systems are presented as highly functional carriers for therapeutic agents, improving the solubility, stability, and bioavailability of encapsulated bioactive substances, enabling controlled release, protection against oxidative and enzymatic degradation, as well as efficient penetration through skin layers. In addition, processes such as ultrasonic sonication, ultraviolet sterilization, and lyophilization are discussed, since they may significantly affect the physical and chemical properties of liposomes and the stability of encapsulated molecules. Furthermore, the development of advanced wound-treatment systems in the form of biopolymeric films was considered, where pullulan stands out as a highly biocompatible and non-toxic matrix. The Introduction emphasizes the need for chemical modifications of pullulan, such as esterification, in order to overcome deficiencies in mechanical properties

and ensure stable integration of lipid carriers. Such an integrated approach, namely the combination of modified biopolymers and liposomal systems, establishes the scientific basis for creating functional materials that actively support tissue regeneration and provide barrier protection against external stressors.

Within the Aims of the Research, the general objective of the doctoral dissertation is stated as the development, characterization, and investigation of multifunctional liposomal and biopolymeric systems for the controlled release of silibinin and *C. coggygia* extract, as well as the evaluation of their physicochemical, structural, mechanical, and biological properties for potential pharmaceutical and biomedical applications. The specific objectives of this dissertation include the preparation and post-process modification of silibinin-loaded liposomes (using ultrasound waves, ultraviolet radiation, and lyophilization), their detailed physicochemical and rheological characterization, as well as spectroscopic analysis of molecular interactions between the active substance and the lipid bilayer. The research further includes the extraction of biologically active compounds from *C. coggygia* and characterization of the obtained extract, the chemical modification of pullulan by esterification for the synthesis of pullulan-isononanoate, and the development of composite films through the incorporation of liposomal vesicles containing silibinin and smoke tree extract into polymer matrices. The final phase is focused on evaluation of the biological activity of silibinin-loaded liposomes (antioxidant, anti-inflammatory, and cytotoxic effects on the HaCaT cell line) and examination of the functional properties of the films (mechanical resistance, swelling, and contact angle), as well as their antioxidant effect, to confirm the potential of the developed systems as advanced platforms for controlled release of natural bioactive components.

The Experimental Part included all materials and methods used in the research work: the ultrasound-assisted extraction method, determination of extraction yield, methods for determining the total flavonoid and polyphenol content in *C. coggygia* extract, the method for preparing silibinin-loaded liposomes, as well as liposomes containing silibinin/*C. coggygia* extract, methods for modifying silibinin-loaded liposomes using an ultrasonic probe and ultraviolet irradiation, the method of lyophilization of silibinin-loaded liposomes, measurement of encapsulation efficiency, determination of particle size, size distribution, and zeta potential, as well as monitoring the stability of liposomes with encapsulated active compounds (dynamic light scattering, DLS method), determination of viscosity, density, and surface tension of liposomal dispersions containing encapsulated silibinin, the method of pullulan modification (esterification), determination of the efficiency of pullulan modification using nuclear magnetic resonance spectroscopy (NMR), determination of the degree of esterification of pullulan by potentiometric titration, determination of phase transitions of pullulan using differential scanning calorimetry (DSC), the method for preparation of biopolymeric films based on modified pullulan, as well as modified pullulan containing incorporated liposomes with active compounds (silibinin and smoke tree extract), the method of chemical composition analysis of the obtained films, liposomes, extracts, and starting components using Fourier-transform infrared spectroscopy (FTIR), the method for determining the contact angle of the obtained films, moisture content, swelling degree, and total soluble matter, optical microscopy, the method for determining mechanical properties of the films, as well as methods for testing antioxidant activity (ABTS and DPPH assays) of the obtained films, liposomes, and extract. In addition, methods performed on the HaCaT cell line were described (approval of the Ethics Committee of the Institute for the Application of Nuclear Energy – INEP, University of Belgrade, No. 0203-07-013/007/2025, dated February 14, 2025), which included examination of the cytotoxic, antioxidant, and anti-inflammatory potential of the developed and modified silibinin-loaded liposomes. The methods included the MTT assay, H2DCFDA assay, and ELISA assay. All statistical tools and software used for statistical data processing were also listed.

In the chapter Results and Discussion, the results of characterization of the developed liposomal systems containing encapsulated silibinin (before and after modifications) are first presented. It was determined that multilamellar liposomes (MLV) showed the highest silibinin encapsulation efficiency of approximately 89.7%, while lyophilized samples had the lowest value (~62.5%) due to disruption of bilayer integrity during the freezing process. Examination of the physicochemical properties of silibinin-loaded liposomes showed that ultrasound treatment significantly reduced particle diameter to approximately 277 nm, whereas UV irradiation did not significantly affect size or surface charge, confirming the protective role of the lipid bilayer. Monitoring of colloidal stability during 60 days of storage indicated a gradual increase in liposome diameter in all formulations due to aggregation and vesicle fusion processes. Specifically, silibinin-loaded liposomes stored at 4°C showed a significant increase in particle size and polydispersity index, as well as a decrease in the absolute value of zeta potential, indicating partial aggregation, reduced homogeneity, and moderate physical instability, while lyophilized and UV-irradiated formulations showed relatively better stability compared with multilamellar and small unilamellar particles. Rheological characteristics of silibinin-loaded liposomes showed low viscosity and moderate surface tension, while UV irradiation and

ultrasound treatment reduced density and suspension stability, indicating increased fluidity and potential instability. This emphasizes the need for additional viscosity modifiers to maintain homogeneity and control particle size during storage.

FTIR spectroscopy showed that UV irradiation causes subtle structural changes in phospholipid liposomes and their multilamellar versions containing silibinin, mainly through oxidation and formation of hydroperoxide and aldehyde functional groups, while ester carbonyl groups remained stable. In contrast, according to FTIR spectra, ultrasonic modification did not affect the characteristics of silibinin-loaded liposomes, indicating preservation of integrity and molecular interactions while simultaneously reducing particle size. Raman spectra of multilamellar liposomes containing silibinin showed efficient encapsulation of the active substance, while UV irradiation caused structural changes in the lipid matrix, whereas ultrasound treatment did not disturb the chemical structure of silibinin-loaded liposomes due to its antioxidant action. The antioxidant potential of silibinin-loaded liposomes was preserved after UV irradiation, reduced after lyophilization due to damage of the lipid matrix, while ultrasound treatment improved antioxidant activity due to increased surface area caused by the presence of small particles.

Analyses of bioactive films based on pullulan-isononanoate confirmed that incorporation of liposomes containing silibinin/smoke tree extract has a plasticizing effect and enables controlled release of antioxidants. Ethanolic extract obtained from the woody part of the smoke tree plant showed a high content of total polyphenols (188.92 mg gallic acid equivalents/g plant material) and total flavonoids (70.76 mg catechin equivalents/g plant material), confirming that this plant material is a valuable source of phenolic compounds, while the extraction yield was 6%, indicating the efficiency of the applied procedure.

Based on NMR analyses, successful esterification of pullulan with 3,5,5-trimethylhexanoic acid chloride (isononanoyl chloride) was confirmed, resulting in a modified polymer with preserved polysaccharide chain structure and approximately 0.81 substituted hydroxyl groups per anhydroglucose unit. DSC analysis showed that esterification of pullulan leads to a significant decrease in phase transition temperature ($\sim 61^{\circ}\text{C}$) and increased flexibility of polymer chains, with the occurrence of thermal degradation at around 207°C .

The determined encapsulation efficiency ($\sim 87.1\%$) indicates that most polyphenolic compounds from smoke tree extract, together with silibinin, were successfully incorporated into liposomal vesicles, thereby ensuring their stability, protection against degradation, and potentially improved bioavailability. DLS analysis results indicate significant changes in the size of liposomes containing silibinin/smoke tree extract during storage (increase up to day 30, followed by partial decrease), suggesting limited physical stability, but also time-dependent potential to act as surface depots for controlled release of bioactive compounds. During 60 days of storage, the polydispersity index (PDI) varied between 0.250 and 0.360, indicating variable heterogeneity, while zeta potential values remained within the range of moderate stability, indicating satisfactory electrostatic repulsion and preserved colloidal stability.

FTIR analysis shows that the chemical structure of pullulan-isononanoate was preserved during film formation, while discrete changes in the $-\text{OH}$ and $\text{C}-\text{O}-\text{C}$ regions indicated interactions with liposomes and bioactive compounds, potentially contributing to better molecular cohesion and improved film integrity. Optical microscopy showed that the highest level of homogeneous liposome integration into films was achieved with 25% liposomal fraction relative to polymer, while higher concentrations led to aggregation and reduced homogeneity, correlating with a decrease in mechanical stability of the films.

Contact angle measurements showed that the film was moderately lipophilic (79.5°), while the addition of liposomes gradually reduced hydrophobicity ($69.3-63.1^{\circ}$), except at the highest liposome content, where aggregation caused an increase to 80.6° , indicating a variable influence of liposomes on surface properties of the films.

Moderate addition of liposomes slightly reduced swelling capacity and total soluble content, with a moderate increase in moisture, while at high liposome content, aggregation caused microvoids, leading to increased water uptake and swelling, with a gradual increase of soluble matter due to partial disruption of the polymer matrix.

Addition of liposomes into pullulan-isononanoate-based films led to reduced tensile strength and elongation at break, with this effect increasing at higher liposome contents. This indicates the formation of heterogeneity and possible phase separation within the polymer network.

Encapsulation of liposomes containing silibinin/smoke tree extract into films significantly increased antioxidant potential in a concentration-dependent manner, demonstrating synergistic action of silibinin and active compounds from smoke tree and ensuring controlled release and stability of polyphenolic compounds. Finally, biological potential tests on HaCaT cells demonstrated that silibinin-loaded liposomes possess significant antioxidant, anti-inflammatory, and cytoprotective activity without exhibiting toxic effects, thereby confirming the justification for potential application of these systems in biomedicine.

In the chapter Conclusions, the most important conclusions derived from the investigations presented in the previous chapter are listed. Based on the conducted research within the doctoral dissertation, conclusions were drawn confirming that silibinin and *C. cogggyria* extract were successfully encapsulated into multilamellar liposomes, significantly improving their stability, solubility, and biological availability while enabling controlled release. Detailed analysis of post-encapsulation treatments showed that lyophilization is suitable for long-term storage and preservation of the therapeutic function of silibinin-loaded liposomes, whereas UV irradiation is not recommended due to disruption of bilayer integrity and increased cytotoxicity. A special contribution is the successful chemical modification of pullulan by esterification with isonanoyl chloride, yielding a branched biopolymer with improved flexibility and thermomechanical properties. Incorporation of liposomes containing silibinin/smoke tree extract into this polymer matrix enabled the development of multifunctional bioactive films. Finally, the research confirms the potential of these composite materials for biomedical applications, particularly as advanced wound dressings, laying the foundation for future studies of synergy between active components and clinical applications.

Thereafter, in the chapter References, all sources used during the preparation and writing of the doctoral dissertation are listed.

In the chapter Supplementary Material, FTIR and Raman spectra of phospholipids (Phospholipon 90G) used for liposome preparation, silibinin, silibinin encapsulated in liposomes, and extract are presented, as well as results of spectral deconvolution, structural formulas of phosphatidylcholine, silibinin, pullulan, 3,5,5-trimethylhexanoic acid, modified pullulan, and their FTIR and NMR spectra, graphical presentation of H2DCFDA assay results for empty liposomes, graphical presentation of MTT assay results used to examine cytotoxicity of lipid carriers themselves on skin cells (HaCaT), as well as graphical presentation of measurements of basal levels of key proteins involved in inflammatory processes within skin cells in the presence of empty liposomes.

3. EVALUATION OF THE DISSERTATION

3.1. Modern Relevance and Originality

The doctoral dissertation of candidate Amjed Karkad addresses a highly relevant scientific field concerning the development of advanced delivery systems for biologically active plant-derived compounds, combining the principles of green chemistry, nanotechnology, and pharmaceutical engineering. The modern relevance of the work is reflected in its response to key challenges of contemporary biomedicine, such as the low solubility and instability of natural antioxidants. The use of silibinin and extract of the plant *C. cogggyria* as active compounds is in line with global trends toward natural, non-toxic, and bioactive therapeutic agents and systems.

The originality and scientific contribution of the dissertation are clearly defined through several innovative segments:

- Innovative chemical modification: For the first time, direct esterification of the polysaccharide pullulan with isonanoyl chloride was successfully performed. The obtained pullulan-isononanoate represents a new biopolymer exhibiting modified water solubility, enabling the formation of flexible and biocompatible films without the use of toxic solvents.
- Systematic analysis of post-encapsulation treatments: The dissertation presents detailed monitoring of the effects of ultrasound treatment, UV irradiation, and lyophilization on the structural integrity of liposomes. Particularly significant is the application of advanced spectral deconvolution within FTIR analysis, as well as analysis of Raman spectroscopy data for identification of subtle changes in the lipid bilayer after treatment.
- Hybrid composite systems: Originality is also reflected in the creation of a complex multifunctional platform-composite biopolymeric films into which liposomes containing biologically active compounds were incorporated. This “system within a system” enables dual protection of active compounds and precisely controlled release kinetics.
- Comprehensive biological validation: The scientific value of the work was further confirmed through in vitro tests on HaCaT cells, where it was demonstrated that the developed silibinin-loaded liposomes not only efficiently suppress oxidative stress and inflammatory mediators (COX-2, IL-1 β , and MIF), but also exhibit absence of cytotoxicity as a prerequisite for biocompatibility, thereby establishing a potential foundation for a new generation of “smart” wound dressings.

The obtained results possess a high degree of originality and contribute to the fundamental understanding of interactions between polymer matrices and lipid carriers, which qualifies this dissertation as a significant advancement in contemporary materials science.

3.2. Review of the Referenced and Utilized Literature

In the doctoral dissertation of candidate Amjed Karkad, a doctoral student at the Faculty of Technology and Metallurgy, University of Belgrade, 184 bibliographic references are cited, all of which are relevant to the field of research and indicate the significance of the investigated subject matter. The cited literature consists predominantly of more recent references. At the same time, the presence of older references can also be observed, containing the fundamental theoretical concepts upon which the research is based. Accordingly, it may be concluded that the studies presented in the cited references were useful in establishing the methodology of the experimental work, as well as during the analysis and discussion of the obtained results.

3.3. Description and Adequacy of the Applied Scientific Methods

The results presented within this doctoral dissertation were obtained through the application of modern preparative and analytical instrumental methods, according to original or modified procedures from relevant scientific literature. The extract of the plant *C. coggygia* was obtained using ultrasound-assisted extraction, while extraction efficiency was evaluated through determination of extraction yield (dry matter measurement), total polyphenol content (Folin-Ciocalteu assay), and total flavonoid content (spectrophotometric assay with aluminum chloride).

Encapsulation of silibinin and smoke tree extract into liposomes was carried out using the proliposome method, which represents a reliable approach for obtaining stable phospholipid vesicles. Comprehensive physicochemical characterization of the obtained encapsulates was performed through determination of encapsulation efficiency (EE), followed by application of the dynamic light scattering (DLS) method for determination of particle size, polydispersity index (PDI), and zeta potential.

Structural changes and molecular interactions were monitored using advanced spectroscopic techniques, namely FTIR and Raman spectroscopy, with the use of FTIR spectral deconvolution for precise band analysis.

The biological potential of the systems was examined through a series of in vitro tests. Antioxidant activity of silibinin-loaded liposomes, liposomes containing silibinin/smoke tree extract, as well as the developed films, was evaluated using the ABTS and DPPH methods, while the cytotoxic profile of silibinin-loaded liposomes was determined by the MTT assay on the HaCaT cell line.

The anti-inflammatory activity of silibinin-loaded liposomes was monitored by measuring levels of pro-inflammatory markers (COX-2, IL-1 β , and MIF) using a cell-based ELISA method on cells treated with bacterial lipopolysaccharide (LPS). Antioxidant activity of silibinin-loaded liposomes was also assessed by measuring intracellular reactive oxygen species levels in the HaCaT cell line using the H2DCFDA assay.

Chemical modification of pullulan into pullulan-isononanoate was confirmed by nuclear magnetic resonance (NMR) and FTIR spectroscopy.

The physical properties of the developed films, including mechanical resistance (universal tensile testing machine), surface wettability (static contact angle), and thermomechanical characteristics (determination of phase transition temperature, T_g), were examined according to standardized protocols.

Statistical processing of all experimental data was performed using one-way analysis of variance (ANOVA) and Tukey's or Duncan's test, thereby ensuring high reliability and reproducibility of the presented results.

3.4. Applicability of the Achieved Results

The results achieved within this doctoral dissertation provide a potentially important basis for practical application in the fields of biomedicine, pharmaceutical technology, and cosmetology, primarily through the development of advanced hybrid systems for local therapy (medicine and pharmacy) or treatment (cosmetics).

Optimized composite films based on the innovative pullulan-isononanoate with incorporated liposomes containing biologically active compounds may represent a potential basis for the production of "smart" wound dressings that provide simultaneous mechanical protection and controlled release of silibinin and smoke tree extract at the target site, which may significantly accelerate tissue regeneration and prevent inflammatory processes.

In addition, implementation of the proliposome method and lyophilization procedures offers the pharmaceutical industry efficient solutions for stabilization of poorly soluble natural antioxidants, while the demonstrated non-toxicity and photoprotective activity of the developed formulations open possibilities for their application in the production of modern dermatological products.

Finally, the successful synthesis of the new biopolymer pullulan-isononanoate provides a methodological and material framework for further design of biocompatible functional materials, whereby this research offers potential technological solutions for the modern development of multifunctional delivery systems for biologically active plant-derived compounds.

3.5. Evaluation of the Candidate's Achieved Ability for Independent Scientific Work

During the preparation of the doctoral dissertation at the Faculty of Technology and Metallurgy, University of Belgrade, Amjed Karkad distinguished himself as an exceptionally independent and creative researcher. His work is characterized by an innovative approach to experimental methods and a high degree of critical judgment in the analysis of the obtained data.

His ability to interpret complex results systematically and scientifically, together with his significant contribution to the advancement of science through additional research, leads the Committee to the opinion that the candidate is fully qualified for independent scientific research work.

4. ACHIEVED SCIENTIFIC CONTRIBUTION

4.1. Overview of the Achieved Scientific Contributions

Within this doctoral dissertation, a significant scientific contribution has been achieved in the field of development and characterization of multifunctional systems for controlled release of plant-derived bioactive compounds, with special emphasis on the combination of liposomal carriers and biopolymeric films.

The most important scientific contributions may be summarized as follows:

- Novel liposomal systems for silibinin encapsulation were developed using the proliposome method, with systematic investigation of the influence of post-encapsulation treatments (ultrasound treatment, UV irradiation, and lyophilization) on their physicochemical and biological properties. This enabled a better understanding of the stability, particle size, surface charge, encapsulation efficiency, and biological potential of the developed silibinin-loaded liposomes after exposure to various external factors.
- A comprehensive physicochemical characterization of silibinin-loaded liposomes was performed, including analysis of size, polydispersity index, zeta potential, rheological properties, and stability over time, as well as evaluation of the cytotoxic, antioxidant, and anti-inflammatory potential of the obtained encapsulates, thereby establishing the suitability of these systems for further potential application in biomedical formulations.
- For the first time, a pullulan derivative – pullulan-isononanoate was synthesized and thoroughly characterized, thereby achieving modification of the hydrophilic-hydrophobic balance of the polymer. This approach enabled improvement of the mechanical, thermal, and surface properties of the films, as well as their compatibility with lipid carriers.
- Composite biopolymeric films based on modified pullulan with incorporated liposomes containing silibinin/smoke tree extract as biologically active components were developed, representing an innovative platform for controlled release of bioactive substances. Their mechanical properties, hydrophobicity, swelling ability, solubility, microstructure, and antioxidant capacity were investigated. It was demonstrated that encapsulation of silibinin and smoke tree extract into liposomes, followed by their incorporation into a biopolymeric matrix, significantly improves the stability and controlled release of silibinin and polyphenols from smoke tree extract, thereby increasing their biological efficiency.
- The biological potential of the developed films was investigated, including antioxidant activity during release in a simulated medium over time, where it was shown that the formulations may possess significant potential for dermal application.

This dissertation contributes to the potential development of new functional materials for biomedical, pharmaceutical, or cosmetic applications through the integration of natural bioactive compounds, liposomal

carriers, and modified biopolymeric matrices, opening possibilities for further research in the field of controlled release and advanced therapeutic systems.

4.2. Critical Analysis of the Research Results

The results obtained within this doctoral dissertation indicate a high potential of the developed liposomal and biopolymeric systems for controlled release of bioactive components, while at the same time revealing certain limitations that should be critically considered.

The achieved high encapsulation efficiency of silibinin in liposomes confirms the appropriate selection of the proliposome method, as well as optimal process conditions. However, variations in particle size and polydispersity index values after different treatments (ultrasound treatment, UV irradiation, and lyophilization) indicate the sensitivity of the system to external factors. In particular, lyophilization, although significantly improving long-term stability, may lead to aggregation and partial alteration of liposome structure, which can potentially affect their functionality after rehydration. The results of dynamic light scattering (DLS) show a relatively narrow particle size distribution in freshly prepared samples, indicating good system homogeneity. Nevertheless, during storage, changes in size and zeta potential were observed, suggesting that long-term colloidal stability may be limited in the absence of additional formulation stabilization, which was the case with the developed silibinin-loaded liposomes. Chemical characterization by FTIR and Raman spectroscopy confirmed successful encapsulation and interactions between silibinin and the lipid phase. However, interpretation of these results may be complicated by the overlap of characteristic peaks, which requires additional analytical techniques for a more precise definition of interactions at the molecular level. The synthesis of pullulan-isononanoate represents a significant contribution, since it enabled the production of films with modified solubility and improved mechanical properties. However, the degree of esterification and its reproducibility may represent a challenge, considering the complexity of the reaction system and possible variations in synthesis conditions. This may affect the consistency of the properties of the obtained films. Incorporation of liposomes containing silibinin/smoke tree extract into the biopolymeric matrix showed a positive effect on the controlled release of bioactive substances. However, possible non-uniform distribution of liposomes within the film and their potential aggregation may lead to local variations in film properties, affecting the reproducibility of the results. Biological studies demonstrated significant antioxidant and anti-inflammatory activity, as well as the prerequisite for satisfactory biocompatibility, i.e., absence of cytotoxicity of silibinin-loaded liposomes. However, the results were obtained under *in vitro* conditions, which limit direct extrapolation to *in vivo* systems. The lack of studies on more complex biological models represents one of the key limitations of the work. In addition, although controlled release of bioactive components was demonstrated, a detailed kinetic analysis of release was not fully elaborated, leaving room for further research aimed at more precise modeling of diffusion and degradation mechanisms. Overall, the results of this dissertation represent a significant step in the development of advanced systems for the delivery of bioactive substances, while simultaneously indicating the need for further optimization of formulations, expanded biological studies, and a deeper understanding of the mechanisms influencing stability and release of active components.

4.3. Verification of Scientific Contributions

Within his doctoral thesis at the Faculty of Technology and Metallurgy, University of Belgrade, Amjed Karkad has focused on the extraction of bioactive compounds from the plant *C. coggygria* and the encapsulation of the obtained extract and silibinin into carrier systems. In addition, the research work of Amjed Karkad is related to the physicochemical characterization of the obtained encapsulates using modern analytical and instrumental techniques.

To date, the candidate has published two scientific papers in international journals, namely one in an international journal of exceptional value (M21a) and one in a top-ranked international journal (M21), as well as four full papers presented at international conferences (M33) and two abstracts presented at international conferences (M34).

Below is the list of publications (published papers) by candidate Amjed Karkad produced during the preparation of the doctoral dissertation entitled "Liposomes and Bio-Polymeric Films for Efficient Controlled Release of Silibinin and *Cotinus coggygria* Scop. Extract."

Category M21a:

1. **Amjed Abdullah Karkad**, Andrea Pirković, Milena Milošević, Bojan Stojadinović, Katarina Šavikin, Aleksandar Marinković, Aleksandra Jovanović, 2024. Silibinin-Loaded Liposomes: The Influence of Modifications on Physicochemical Characteristics, Stability, and Bioactivity Associated with Dermal Application. *Pharmaceutics* 16(11), 1476. <https://doi.org/10.3390/pharmaceutics16111476>. IF (5) = 5.8

Category M21:

1. **Amjed Abdullah Karkad**, Aleksandar Marinković, Aleksandra Jovanović, Katarina Simić, Stefan Ivanović, Milena Milošević, Tamara Erceg, 2026. Design and Optimization of Pullulan-Isononanoate Films with Bioactive-Loaded Liposomes for Potential Biomedical Use. *Polymers* 18(2), 305. <https://doi.org/10.3390/polym18020305>. IF (5) = 5.2

5. VERIFICATION OF THE ORIGINALITY OF THE DOCTORAL DISSERTATION

Pursuant to the Regulations on the Procedure for Verifying the Originality of Doctoral Dissertations Defended at the University of Belgrade (Official Gazette of the University of Belgrade, No. 204 of June 22, 2018), the originality of the doctoral dissertation of candidate Amjed Karkad, entitled “Liposomes and Bio-Polymeric Films for Efficient Controlled Release of Silibinin and *Cotinus coggygria* Scop. Extract”, was checked using the iThenticate software on March 19, 2026.

The determined similarity index was 6%. This percentage is the result of common expressions, *i.e.*, the use of technical terms and data related to the studied topic, names of the methods used and their abbreviations, personal names, instruments, as well as the candidate’s own statements. Part of the similarity refers to previously published results of the candidate’s research, which originated from his dissertation.

Based on all of the above, the Committee considers that the doctoral dissertation of candidate Amjed Karkad is original, and that academic citation rules have been fully respected; therefore, the prescribed procedure for preparation of its defense may proceed.

6. CONCLUSION AND PROPOSAL

Based on the data and results previously presented, the Committee concludes that the doctoral thesis of candidate Amjed Karkad represents a significant contribution to the scientific field to which it belongs, as supported by papers published in reputable international journals. The subject matter and objectives defined in the report on the scientific validity of the dissertation topic have been fully achieved.

Therefore, the Committee thinks that the present doctoral dissertation satisfies all necessary criteria, and that during the course of the scientific research work, the candidate demonstrated a high degree of independence, creativity, and responsibility.

Having in mind the scope, complexity, and scientific contribution of the achieved results in the field of Chemical Sciences, the Committee proposes that the Teaching and Scientific Council of the Faculty of Technology and Metallurgy, University of Belgrade, accept this report and, together with the submitted doctoral dissertation of candidate Amjed Karkad entitled:

“Liposomes and Bio-Polymeric Films for Efficient Controlled Release of Silibinin and *Cotinus coggygria* Scop. Extract”

In Belgrade, April 14, 2026.

MEMBERS OF THE COMMITTEE

Professor Dr. Suzana Dimitrijević-Branković, Full Professor
University of Belgrade, Faculty of Technology and Metallurgy

Professor Dr. Antonije Onjia, Full Professor
University of Belgrade, Faculty of Technology and Metallurgy

Dr. Aleksandra Jovanović, Senior Research Associate
University of Belgrade, Institute for the Application of Nuclear Energy – INEP