BULGARIAN ACADEMY OF SCIENCES
INSTITUTE OF ORGANIC CHEMISTRY WITH CENTRE OF PHYTOCHEMISTRY

Extended habilitation summary of scientific contributions

Dr. Ivalina Ognyanova Trendafilova
Laboratory of Oganic reactions on microporous silica materials
Institute of Organic Chemistry with Centre of Phytochemistry
Bulgarian academy of sciences

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I. Introduction

The need to overcome a number of significant limitations associated with the effective application of biologically active compounds (BACs) of natural origin - such as low solubility in biological media, rapid metabolism, chemical instability, potential adverse side effects, and the requirement for frequent administration - has stimulated increasing interest from both the academic community and the pharmaceutical industry in the development of innovative carriers and delivery systems.

Among the wide range of investigated materials, mesoporous silicates have emerged as particularly promising platforms for the development of advanced therapeutic and diagnostic systems, enabling improved solubility and bioavailability, controlled release, and targeted delivery of biologically active compounds (BACs), as well as the immobilization of biomolecules such as genes, proteins, and enzymes, including applications in regenerative medicine. They are characterized by exceptionally high specific surface area, large pore volume, and well-organized porous architecture, which is a premise for efficient loading with a broad spectrum of biologically active molecules. A key advantage of these materials lies in the possibility for precise control over pore size and particle morphology, providing opportunities to tailor the rate and mechanism of release of the incorporated substances. Furthermore, the presence of reactive silanol groups on the surface of silica particles enables their targeted functionalization with various organic moieties and/or metal nanoparticles, leading to enhanced affinity between the carrier and the adsorbate and facilitating the development of targeted and stimuli-responsive delivery systems.

In comparison with conventional polymeric and lipid-based carriers, mesoporous silicates exhibit higher structural stability, greater loading capacity, and the ability to enhance the solubility and bioavailability of poorly water-soluble BACs. They provide a more homogeneous distribution of active substances within the matrix and allow improved control over their release kinetics. Furthermore, silica materials are characterized by good biocompatibility and high chemical and thermal stability, enabling their application across a wide range of temperatures and pH values, including in the presence of organic solvents. Owing to this combination of unique properties, mesoporous silica materials are considered an exceptionally promising platform for the development of advanced drug delivery systems.

The first reports in the international scientific literature on the application of mesoporous silica materials as carriers for pharmaceutical compounds date back to 2001, whereas research in this field in Bulgaria commenced in 2011 at the Institute of Organic Chemistry with the Centre of

Phytochemistry, Bulgarian Academy of Sciences, under the leadership of Prof. Dr. Margarita Popova. I joined this research team in 2014, focusing my scientific activity on the rapidly evolving interdisciplinary field of nanomedicine. The primary aim of the conducted studies was the development of innovative theranostic systems - combining therapeutic and diagnostic functionalities - for socially significant diseases, through the incorporation of anti-inflammatory and antimicrobial agents, as well as potential chemotherapeutic compounds of natural and synthetic origin, into nanoscale composite carriers based on mesoporous silicates. Currently, the research group continues to actively work on the synthesis, modification, and application of mesoporous silica materials, as well as magnetic-silica and organic–inorganic nanocomposites, with a particular focus on their utilization as efficient platforms in modern drug delivery systems.

In this context, the development of novel approaches for the synthesis, modification, and functionalization of mesoporous silicate carriers emerges as a significant scientific challenge with the potential to yield substantial fundamental and applied contributions. The research presented in the current habilitation thesis is focused on the development of innovative strategies for the synthesis, structural and chemical modification of mesoporous silicate materials, as well as on the systematic investigation of their application as carriers for biologically active compounds. The results obtained contribute to a deeper understanding of the relationships between synthesis conditions, the structural and textural characteristics of the materials, and their functional properties, which is of critical importance for the design and development of a new generation of highly effective materials with potential applications in biomedicine and pharmaceutical practice.

The present habilitation report encompasses the most significant aspects of my research activities following the completion of my doctoral degree at the Institute of Organic Chemistry with Centre of Phytochemistry, BAS in 2018. It is based on a total of 16 scientific publications dedicated to the development of innovative delivery systems for biologically active compounds of natural origin, utilizing mesoporous silicate materials and their composites. Five of these publications have been accredited as a habilitation work (indicator “В”) [pub. No. 1–5], while the remaining 11 publications have been classified under indicator “Г” [pub. No. 6–16]. The conducted research is in the fields of materials science, medicine, and pharmacy, with contributions of both fundamental and applied scientific significance. The main scientific and applied contributions of these works can be summarized within the following key directions:

- Development of innovative and highly efficient systems enabling targeted delivery and controlled release of BACs, based on composite silica carriers. The research in this area is focused on the development of procedures for the synthesis of hybrid carriers based on mesoporous silicates, their efficient loading with biologically active compounds, and their potential applications in diagnostics, pharmacy, and medicine;
- Establishment of the relationship between synthesis conditions and the physicochemical properties of the resulting materials, achieved through comprehensive characterization of their structural, textural, and surface properties using advanced physicochemical techniques (low-temperature nitrogen physisorption, X-ray diffraction, electron microscopy, UV, IR, and NMR spectroscopy, and thermogravimetric analysis);
- Investigation of the interactions between biologically active molecules and polymer coatings with the surface of both modified and parent silica carriers, employing IR and NMR spectroscopy, thermogravimetric analysis, and quantum-chemical calculations;
- Investigation of the pharmacokinetics, biological activity on selected cell lines, and antioxidant properties of the developed delivery systems.

II. Key Contributions

The obtained results are in the field of development of innovative methods for the synthesis of novel nanoscale composites based on modified silica particles with controlled porous structures and their application as carriers in delivery systems for natural biologically active compounds.

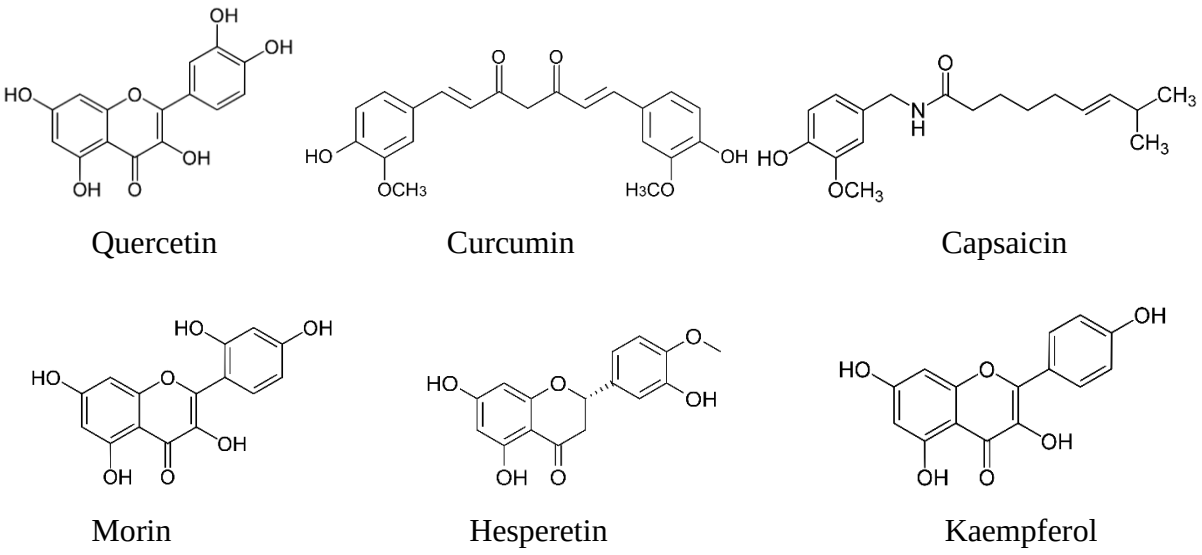
In recent years, natural flavonoids have attracted considerable interest within the scientific community due to their broad therapeutic potential. Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one, Scheme 1) is one of the most widespread flavonols in nature, a potent antioxidant, and a major dietary flavonoid. It is well established that quercetin exhibits antitumor, antiviral, and antimutagenic activities, as well as the ability to inhibit lipid peroxidation. However, the clinical application of quercetin's therapeutic potential is severely limited by its unfavorable physicochemical and pharmacokinetic properties. Quercetin is characterized by very low water solubility and chemical instability, resulting in poor bioavailability. One potential strategy to overcome these limitations is the development of carriers for optimized oral, parenteral, local, or targeted delivery of quercetin. Considering that quercetin, as a strong antioxidant, primarily exerts its activity through metal ion chelation (Fe^{2+}, Fe^{3+}, Cu^{2+}), and that zinc, as a chelating agent, can form complexes with a wide range of biologically active compounds while also playing a key

role in wound healing and collagen synthesis, the quercetin–zinc combination is particularly promising for incorporation into dermal formulations. Based on these considerations, a novel approach was developed for optimized dermal delivery of quercetin via its incorporation into zinc-modified mesoporous silicate materials.

The main results can be summarized as follows:

- A mesoporous silica type SBA-15 was synthesized. It was selected for its suitable pore structure (ordered 2D hexagonal mesostructure), pore size (6 nm), particle size (1–2 μm), and high specific surface area (870 m^2/g).
- The optimal conditions for the obtained material's post-synthetic modification with varying amounts of Zn (2 or 4 wt.%) were established. It was demonstrated that zinc is incorporated into the silica walls both as ions and as zinc oxide nanoparticles (<5 nm) in the sample modified with 2 wt.%, whereas in the sample modified with 4 wt.% the formation of larger zinc oxide nanoparticles (~20 nm) was also observed. These nanoparticles are either encapsulated within the channels or located on the external surface of the silica particles.
- Quercetin was successfully loaded into both the parent and Zn-modified silica carriers (over 40 wt.%) using the incipient wetness impregnation method. It was demonstrated that this modification technique results in the incorporation of the anhydrous form of quercetin within the silica, due to the recrystallization of the biologically active compound in ethanol during the loading procedure. This was further confirmed by recrystallization of the starting quercetin dihydrate in ethanol. It was established that the hydrated and anhydrous forms of quercetin can be distinguished by X-ray diffraction (XRD) and FT-IR spectroscopy.
- For the first time, the formation of a Zn–quercetin complex on the surface of silica was observed using FT-IR spectroscopy.
- The in vitro release study of the loaded quercetin at pH 5.5 (typical for dermal delivery systems) demonstrated a prolonged release from the Zn-modified samples compared to the parent SBA-15, which is attributed to the interactions between quercetin molecules and the metal/metal oxide particles.
- Comparative cytotoxicity studies demonstrated that quercetin loaded Zn-modified silicate carrier (2 wt.%) exhibits a higher antineoplastic potential against HUT-29 (cutaneous T-cell lymphoma) cells compared to the unencapsulated (pure) biologically active compound.

The high loading capacity and controlled release of quercetin, together with the enhanced antineoplastic activity observed in HUT-29 (cutaneous T-cell lymphoma) cells, indicate that systems based on Zn-modified SBA-15 materials represent highly promising carriers for the dermal delivery of quercetin [1].



Scheme 1. Chemical structure of the used biologically active substances

In addition to the development of dermal delivery systems for flavonoids such as quercetin, considerable attention has been directed toward the design of similar platforms for different classes of natural compounds with proven health-promoting properties. Subsequent studies focused on the development of a dermal delivery system for a combination of two biologically active compounds and the investigation of their potential synergistic effects upon application. For the design of an innovative system containing two natural active compounds, curcumin ((1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione, Scheme 1) and capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide, Scheme 1) were selected as a suitable combination.

Curcumin and capsaicin have been widely used as traditional medicinal agents for centuries. Owing to its low toxicity and significant pharmacological activity, curcumin has been extensively investigated for the treatment of various diseases, including cancer, inflammatory disorders, and diabetes. On the other hand, capsaicin - the principal active component of chili peppers - has been utilized in medicine due to its ability to reduce cholesterol, lipid, and blood glucose levels, as well as for its antioxidant, anti-inflammatory, and analgesic properties. Numerous studies have demonstrated the potential of capsaicin as an antitumor agent against a

wide range of cancers, including those of the breast, lung, prostate, stomach, liver, and bladder. Furthermore, this pungent alkaloid has been shown to enhance nutrient permeability and to act as a co-adjuvant, improving the absorption of curcumin.

The mechanism of action underlying the beneficial biological activities of these two well-known natural compounds has been extensively investigated and documented. However, their clinical application remains significantly limited due to their low aqueous solubility, restricted distribution to target tissues, poor bioavailability, and short half-life resulting from extensive metabolism, as well as the pronounced irritant effect of capsaicin. In our previous studies on quercetin, we demonstrated that these limitations can be effectively overcome through the incorporation of BACs into mesoporous silica carriers modified with metal ions/particles. In the present study, silver was selected as a modifying agent not only because of its well-known antibacterial (antimicrobial) properties and potential beneficial effects on damaged skin, but also due to its ability to form complexes with curcumin and capsaicin. The formation of such complexes is expected to facilitate more efficient loading of these compounds into the pores of the silica matrix and to enable their controlled release. The most significant results obtained are as follows:

- Spherical mesoporous silicate particles with sizes in the range of 5–8 μm, high specific surface area (904 m^2/g), and pore volume (above 0.6 cm^3/g) were synthesized in the presence of two structure-directing agents (templates)—CTAB and Pluronic 123.
- The obtained materials were successfully modified with silver via template-ion-exchange method. The results demonstrated that the modification of the parent mesoporous silica with silver through ion exchange, followed by template removal, yielded materials with larger pore sizes and volumes compared to those produced by the conventionally employed template removal method—calcination. It was established that the applied modification method resulted in materials containing both silver oxide and metallic silver nanoparticles.
- The silver-containing and parent silica carriers were successfully loaded with curcumin, capsaicin, or a mixture of both via the incipient wetness impregnation method from ethanolic solutions. The results demonstrated efficient incorporation of the BACs in all samples (up to 33 wt.%). An exception was observed for the sample loaded with the mixture of both compounds on the unmodified mesoporous silica (22 wt.%), where the lower loading capacity can be attributed to the narrower pores - constricted as a result of the calcination step for template removal - which are easily blocked and limit

molecular diffusion. Interestingly, thermogravimetric (TG) analysis revealed that the unmodified and silver-containing mesoporous silicas loaded with the curcumin - capsaicin mixture exhibited significant mass loss (approximately 15 wt.%) below 100 °C, likely due to solvent loss, which may act as a bridging molecule facilitating the formation of a network between the two bioactive molecules. Such an effect was not observed in single-component formulations. We hypothesize that the formation of a molecular network of capsaicin and curcumin, interconnected via solvent (ethanol) bridges, restricts their penetration into the mesoporous channels, which may explain the lower loading observed even in the unmodified carrier, characterized by narrower pores.

- Spectroscopic data revealed weak interactions of curcumin and capsaicin with the surfaces of both unmodified and Ag-modified carriers. Differences were also observed between the spectra of a physical mixture of curcumin and capsaicin and that obtained from the ethanolic solution. In the latter case, signals attributable to residual ethanol, associated with the two bioactive compounds via hydrogen bonding, were detected, which is consistent with the results from TG analysis.
- The results of *in vitro* release studies of curcumin and capsaicin demonstrate that their incorporation into mesoporous silica carriers leads to a significant enhancement of their solubility at 35 °C in a pH 5.5 buffer, characteristic of dermal formulations. The most pronounced effect was observed for the system based on the Ag-modified silica carrier, which exhibited nearly complete release of the loaded amount (~100%) within the first 15 minutes. In the case of simultaneous loading of both BACs, a slower release profile was observed, with approximately 80% of the loaded compounds released by 120 minutes. This behavior is likely attributable to the formation of a supramolecular network of curcumin and capsaicin molecules, which may hinder the diffusion of the buffer into the pores as well as the subsequent diffusion of the bioactive compounds out of the carrier's pores.
- Alongside the investigation of release profiles, it is important to assess the retention of the bioactive compounds' biological activity after their incorporation into the silica carriers. Accordingly, their antioxidant and antibacterial activities, as well as their cytotoxic potential, were evaluated. It was found that in delivery systems based on unmodified silica, the DPPH radicals scavenging activity (RSA) and their antioxidant activity, as determined by ferric-reducing antioxidant power (FRAP) of loaded

curcumin and capsaicin are preserved. In contrast, for delivery systems based on Ag-modified carriers containing curcumin or a curcumin–capsaicin mixture, the RSA and FRAP values decrease, which may be attributed to additional interactions du to the formation of Ag–curcumin complexes or interactions between curcumin and capsaicin molecules.

- The analysis of the antibacterial activity of the obtained curcumin- and capsaicin-loaded systems demonstrated that the presence of silver in the carrier enhances the antibacterial efficacy of the formulations compared to the pure bioactive compounds against three pathogenic microorganisms: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.
- A comparative study of the cytotoxic potential of free and loaded bioactive compounds demonstrated that the system for the simultaneous delivery of curcumin and capsaicin exhibits IC_{50} values at least 1.5 times lower than those of the free compounds against the HUT-78 cell line (cutaneous T-cell lymphoma). These results support the conclusion that the combination of curcumin and capsaicin exerts a synergistic effect against the tested cancer cell lines.

Considering the excellent results from the antineoplastic, antioxidant, and antibacterial activity assays of the delivery systems based on silver-modified silicate particles loaded with curcumin and capsaicin, it can be concluded that these systems represent promising candidates for potential dermal applications in the treatment of various skin conditions [2].

Alongside the prospects for dermal application, the development of drug delivery systems for oral administration remains one of the main directions in contemporary pharmaceutical research. The design of a quercetin delivery system intended for oral administration requires a tailored approach that considers the specific characteristics and demands of this route of drug administration. For oral formulations, it is essential to protect the biologically active compound from degradation under the low pH conditions of the stomach and to ensure its controlled release. This can be achieved through the use of polymers with pH-dependent solubility. In the systems developed in our studies, the advantage of porous silicates in enhancing the solubility of quercetin in biological media was combined with the protective effect against the aggressive gastric environment provided by suitable polymers. The main results include:

- A spherical mesoporous silica of the KIT-6 type with a particle size of ~45 nm, a high specific surface area (770 m^2/g), and an pore size of 6 nm was synthesized.

- The surface of the silica particles was successfully functionalized with amino groups via a post-synthetic method. The influence of these surface groups on the loading capacity and release rate of quercetin was investigated, as well as their role in the formation of polymeric layers around the quercetin-loaded silica particles.
- To optimize the loading of quercetin onto the amino-functionalized samples, two methods were employed: incipient wetness impregnation and solid-state reaction. Both approaches achieved high loading capacities, with the wet impregnation method yielding a higher loading percentage (33 wt.%) compared to the solid-state reaction (29 wt.%). It was demonstrated that wet impregnation promotes a greater degree of amorphization of the bioactive compound and predominantly localizes it within the pores of the carrier, whereas the solid-state reaction results in the formation of larger quercetin crystallites on the silica surface.
- *In vitro* studies of quercetin release from the amino-functionalized silicates demonstrated a faster release from the sample prepared via wet impregnation compared to that obtained through the solid-state reaction. This effect can be attributed to the enhanced solubility of quercetin resulting from its amorphization and the prevention of the formation of larger, poorly soluble crystals during the wet impregnation process. A weak interaction was observed between quercetin and the NH_2 groups on the silica surface, involving the phenolic moiety of the quercetin molecule.
- Quantum-chemical modeling provided insights into the interactions between quercetin molecules and the functionalized carrier. It was found that the adsorption of quercetin molecules is significantly stronger (more than twofold) on the amino groups of the neutral carrier compared to the ammonium groups of the protonated KIT-6 silica material. In the case of the neutral KIT-6NH_2 carrier, where the amino groups are accessible, the most stable complexes form at one of the phenolic OH groups in ring B of the quercetin molecule, which acts as a proton donor and ensures high loading capacity. In contrast, on the protonated KIT-6NH_2 carrier, quercetin binding is weaker, as the hydrogen bond is formed between the hydrogen atom of the NH_3^+ group and the carbonyl oxygen of the quercetin molecule. This behavior allows drug molecules to bind effectively to the neutral carrier and subsequently be released in a more acidic environment when the carrier becomes protonated.
- It was established that the release of quercetin loaded onto a silica carrier can be further controlled through coating with appropriate polymer layers. For this purpose, a

polyelectrolyte polymer complex consisting of alternating layers of κ-carrageenan/chitosan/κ-carrageenan was constructed around the loaded silica particles. It was demonstrated that, following the formation of the polyelectrolyte layer, the particle size increased from ~45 nm (parent silica) to 50-70 nm, providing evidence of the successful coating of the spherical silica nanoparticles. *In vitro* release studies of quercetin from the amino-functionalized and polymer-coated silicates showed a significant prolongation of the release time from 4 hours to over 24 hours.

- *In vitro tests* on selected cell lines demonstrated that even at high doses, the unloaded pristine and amino-functionalized silica nanoparticles did not induce significant reductions in cell viability in the examined benign HEK-293 (human embryonic kidney), malignant HL-60 (acute myeloid leukemia), and malignant HUT-78 (cutaneous T-cell lymphoma) lines. Comparative studies of the cytotoxic potential of free versus loaded quercetin revealed dose-dependent cytotoxicity and showed that its encapsulation within polyelectrolyte-coated, amino-functionalized silica did not reduce its cytotoxic potential against the HUT-78 cell line. These results demonstrate that the developed nanoscale delivery systems are promising for oral formulation of quercetin with prolonged release [3].

In another orally administered system, silver and magnesium were investigated as potential modifying agents for SBA-16 silica carriers in delivery systems for the polyphenols morin and hesperetin (Scheme 1). Morin (3,5,7,2′,4′-pentahydroxyflavone) and hesperetin (3′,5,7-trihydroxy-4′-methoxyflavanone) also belong to the flavonoid class and, like quercetin, exhibit multiple beneficial effects on human health, including anti-inflammatory, antitumor, antioxidant, antidiabetic, anti-allergic, and cardioprotective properties. In this study, silver (Ag) and magnesium (Mg) were selected as modifying agents due to their potential to influence loading capacity and release kinetics, as well as to contribute to the overall health-promoting properties of the resulting delivery systems. Silver has a long-standing history in medicine, being used in wound healing, reconstructive orthopedic surgery, cardiac devices, catheters, and surgical instruments, whereas magnesium is an essential micronutrient with well-established beneficial effects on human health, acting as a cofactor for over 300 enzymes and regulating muscle contraction, neuromuscular conduction, glycemic control, myocardial contractility, and blood pressure. The main scientific findings of this study can be summarized as follows:

- SBA-16 type silicate particles with an ordered cubic mesostructure and a particle size of 1.5 μm with a narrow particle size distribution were successfully synthesized. The

resulting material is characterized by a high specific surface area (886 m^2/g) and an average pore size of 4.5 nm.

- The silicate was post-synthetically modified with Ag and Mg (4 wt.%), demonstrating that Mg is incorporated into the silicate structure in the form of ions, whereas silver is stabilized both as nanoparticles (<5 nm) within the pores and on the external surface of the carrier.
- A high loading capacity of morin and hesperetin onto both parent and modified mesoporous silicates was achieved via incipient wetness impregnation method. An observed trend indicated enhanced loading efficiency of the bioactive compounds on silicates modified with silver or magnesium, with the exception of the Mg–hesperetin sample. Investigation of the interactions between morin and hesperetin molecules and the surface groups of the pristine and modified carriers revealed that: i) the interaction between the metal particles and hesperetin occurs via the hydroxyl group on ring A or the carboxyl group on ring C of the molecule, whereas interaction of the flavonoid with the silanol groups on the surface of the pristine silicate does not involve charge-transfer interactions; ii) the carbonyl group mediates the interaction of morin with the mesoporous carrier, leading to a weakening of the intramolecular hydrogen bond between the aromatic and pyrone rings within the morin molecule.
- *In vitro* release experiments of the loaded BACs at pH 1.2 (simulating gastric conditions) and pH 6.8 (simulating intestinal conditions) demonstrated improved release profiles of the loaded flavonoids compared to the pure compounds. The release profiles of hesperetin loaded onto either pristine or modified silicates exhibited a biphasic pattern. An initial rapid release of approximately 20% was observed within the first 30 minutes of incubation, followed by a slow and sustained release over the subsequent 6 hours. This behavior can be attributed to the loading of amorphized flavonoid both within the pores and on the external surface of the carrier. Modification of the silica carriers with Ag or Mg did not significantly alter the release profile under different pH conditions. In all tested formulations, complete release of the drug was not achieved within the 6-hour period, likely due to the presence of less soluble crystalline fractions of hesperetin or interactions between hesperetin molecules and the surface functional groups within the bimodal pore structure of SBA-16, which consists of narrow pore entrances and larger cavities, hindering diffusion. In contrast, morin exhibited pH-dependent release behavior. Pure morin was poorly soluble in acidic

medium (only ~20% dissolved over 6 hours at pH 1.2) compared to complete dissolution at pH 6.8 within less than 10 minutes. The slower release of morin from the nanoporous silicates can be attributed to the bimodal pore structure of the SBA-16 carrier. Loading of morin into pristine or Ag- or Mg-modified silicate carriers enhanced its solubility in acidic conditions. Nevertheless, the release from both pristine and modified carriers at pH 1.2 remained incomplete over the 6-hour period. Conversely, at pH 6.8, morin was fully released after 2, 4, and 6 hours from the pristine, Ag-, and Mg-modified carriers, respectively, indicating that appropriate carrier modification can enable controlled release of the loaded bioactive compound.

- The cytotoxicity of morin and hesperetin loaded into pure or modified carriers was evaluated in comparison to the free compounds against both non-malignant and malignant cells: HEK-293 (human embryonic kidney cells), HL-60 (acute promyelocytic leukemia), and U-266 (multiple myeloma). The free morin and hesperetin exhibited concentration-dependent cytotoxic effects against the non-malignant cell line, whereas loading the selected flavonoids into mesoporous silicates reduced toxicity toward healthy cells. The cytotoxic effect of flavonoid-loaded systems against the tumorigenic HL-60 and U-266 cell lines was more pronounced; however, compared to the free compounds, the inhibitory effect on cell proliferation was lower for the loaded flavonoids. The observed lower cytotoxicity of the formulated bioactive compounds is likely attributable to the slower release of the drug. In addition to the reduced antiproliferative activity, the developed nanoporous formulations displayed selectivity toward tumor cells, which makes them less harmful to healthy cells.
- The evaluation of antioxidant capacity demonstrated that loading the flavonoids into both pure and modified silica carriers did not compromise their activity; notably, in systems containing both hesperetin and morin, an enhancement of antioxidant activity was observed.

Therefore, it can be concluded that Mg- and Ag-modified SBA-16 silica particles represent promising carriers for the controlled delivery of bioflavonoids, essential trace elements, and antimicrobial agents [4].

The results of the studies presented above clearly demonstrate that surface modification of silicate carriers plays a key role in the development of effective delivery systems for biologically active compounds (BACs). Such modification enables improved solubility of poorly water-soluble substances, controlled release, and even enhanced therapeutic efficacy

through the incorporation of essential trace elements or antimicrobial agents. These findings provided the basis for our subsequent study, in which three different approaches to silicate surface modification were compared: *in situ* (direct synthesis), post-synthesis wet impregnation, and ion exchange with the template. In this study, the primary focus was to determine the influence of these modification procedures on Mg-modified mesoporous MCM-41 silica and the subsequent use of these materials as carriers for the delivery of the polyphenol kaempferol. Kaempferol (3,5,7,4′-tetrahydroxyflavone, Scheme 1), like other flavonoids, is a potent antioxidant with numerous beneficial effects on human health. The most significant findings of this study can be summarized as follows:

- A mesoporous silicate type MCM-41 was successfully synthesized, it characterized by hexagonally arranged pores with a high degree of ordering. The resulting material exhibited a high specific surface area (952 m^2/g) and a particle morphology combining horseshoe-shaped particles of approximately 1.2 μm with spherical particles of ~100 nm. For the pristine material and the Mg-containing sample obtained via incipient wetness impregnation, the average pore size was determined to be 2.2 nm, whereas the ion-exchanged sample exhibited pores of 2.7 nm, and the directly synthesized sample had an average pore size of 2.6 nm. The larger pore size observed in the ion-exchanged silicate can be attributed to the "soft" conditions used for template removal (pore liberation) through extraction during the magnesium incorporation procedure. In contrast, for the pure silica and the wet-impregnated sample, template removal involved high-temperature calcination, which can lead to pore shrinkage. In the Mg-containing silicate obtained via direct synthesis, the increased pore size is a consequence of the incorporation of magnesium species (ions or oxide nanoparticles) into the silicate framework, inducing partial structural deformation and the formation of larger cavities.
- After modification of the silica with magnesium using the three selected techniques, it was found that the direct synthesis procedure resulted in the incorporation of a higher amount of modifying agent (1.7 wt.%) compared to the post-synthesis methods wet impregnation (0.6 wt.%) and template-ion-exchange (1 wt.%). In the sample obtained via ion-exchange a higher Mg content was observed on the external surface of the silicate compared to the other two samples. This can be explained by the deposition of the modifying agent primarily on the particle surface, as the pores remain inaccessible due to the presence of the template. In contrast, the directly synthesized sample

exhibited the highest amount of magnesium incorporated into the silicate walls, a result of the presence of the modifying agent during the formation of the silica mesophase.

- Kaempferol was successfully loaded into all studied samples, the magnesium modification led to higher loading (~30 wt.%) in all samples compared to the unmodified silica carrier (25 wt.%). Studies of the kaempferol molecules and the carriers' interactions revealed the most pronounced effect for the Mg-silicate system obtained via direct synthesis. This effect can be attributed to the presence of the highest amount of modifying agent in this sample. It was determined that the interaction with the carrier occurs predominantly through the hydroxyl groups on rings B and A of the flavonoid molecule.
- The release profiles of kaempferol were investigated in buffers physiologically relevant for oral administration (pH 1.2 and 6.8) at 37 °C. Experiments in both media showed that pure kaempferol was practically insoluble under these conditions, whereas the release profiles of kaempferol loaded into unmodified or Mg-modified MCM-41 carriers exhibited an initial rapid release of the drug (ranging from 20 to 40% at pH 1.2 and 17 to 22% at pH 6.8) within the first 30 minutes of incubation, followed by a plateau over the subsequent 6 hours. The observed enhancement in drug solubility can be attributed to its amorphization during loading into the silicate pores. The lowest kaempferol release (~20% at pH 1.2 and 5% at pH 6.8) was observed for the Mg-containing sample prepared via wet impregnation, likely due to pore blockage by nanoparticles of the modifying agent during the loading procedure. The highest release of kaempferol was observed for the sample obtained via ion-exchange (~40% at pH 1.2 and 25% at pH 6.8), which is attributed to the formation of larger pores and the generation of more finely dispersed particles of the modifying agent.
- The radical-scavenging activity of kaempferol-loaded unmodified silica and three Mg-modified samples was evaluated against DPPH free radicals. The studies demonstrated that, compared to the pure (unloaded) flavonoid, the Mg-containing sample prepared via ion exchange and loaded with kaempferol exhibited the highest activity, comparable to that of the pure compound. In contrast, the Mg-silicate system obtained via direct synthesis showed a significant reduction in activity, nearly twofold lower than that of the pure flavonoid, which can be attributed to the formation of a Mg-kaempferol complex, as also evidenced by the results indicating the strongest interaction of the flavonoid with this carrier.

Based on these results, it can be concluded that different approaches to silicate modification can yield materials with distinct physicochemical properties, which directly influence their behavior and effectiveness in the construction of delivery systems for bioactive compounds. In the present study, post-synthetic surface modification procedures demonstrated superior performance in producing suitable carriers for oral delivery systems of flavonoids [5].

During the research conducted over the given period, various hybrid materials, including inorganic–inorganic and organic–inorganic composites based on porous silicates, were developed with the aim of applying them in the design of efficient delivery systems for natural and synthetic bioactive compounds. On the base of nanosized Beta zeolite a dual-component drug delivery system was developed for the simultaneous release of silver and sulfadiazine. The study monitored the extent and rate of release of both the drug and silver in a simulated physiological environment, as well as the antibacterial properties of the resulting system [6]. Composite materials of the zeolite–mesoporous silicate type, ZSM-5/KIT-6 and ZSM-5/SBA-15, were synthesized and subsequently functionalized with sulfonic and carboxylic groups. These materials were loaded with verapamil and further coated with a polyelectrolyte multilayer composed of chitosan/κ-carrageenan/chitosan-poli-sulfobetaine. The resulting systems were investigated for their potential application as tools to overcome multidrug resistance (MDR) in cancer cells [7]. Core-shell composites consisting of magnetic nanoparticles embedded in mesoporous silica were successfully synthesized, and their potential as carriers for prednisolone was investigated. The *in vivo* anti-inflammatory activity of the resulting systems was also evaluated [8]. Another type of nanoscale composite material, consisting of magnetic nanoparticles embedded in mesoporous silica (MCM-41), was synthesized, with the optimal conditions for its effective modification with amino or carboxyl groups and subsequent grafting of PEG chains systematically investigated. Following loading of the anticancer therapeutic tamoxifen into the modified and PEGylated nanoparticles, it was found that both the loading efficiency and the drug release profile were highly dependent on the method used to prepare the modified carrier [9]. In curcumin delivery systems based on amino-modified mesoporous silica nanoparticles coated with a polyelectrolyte complex (κ-carrageenan/chitosan), the influence of the pore structure of different carriers (KIT-6 and KIL-2) on the properties of the resulting systems was investigated [10].

Summary of Contributions:

Methods for the synthesis of mesoporous silica materials with predefined properties (particle shape and size, pore geometry) as well as hybrid composites (silica–metal particles, silica–organic

groups, silica–polymer) were developed. Optimal conditions for successful surface functionalization with various groups and for the efficient loading of bioactive compounds (BACs) of natural origin were established. It was demonstrated that incorporation of anti-inflammatory, antimicrobial, and chemotherapeutic agents into modified mesoporous silica materials can enhance their solubility, while the coating with a polyelectrolyte polymer layer around the loaded particles enables targeted delivery and sustained release of the encapsulated BACs, allowing a reduction in dosing frequency while maintaining effective therapeutic concentrations. For the first time, the formation of complexes between flavonoids and metal-functionalized mesoporous silicas was investigated. The results showed that the developed materials possess superior properties as carriers for natural compounds (quercetin, curcumin, capsaicin, morin, hesperetin, kaempferol), enhancing their solubility in physiologically relevant media for both dermal and oral formulations, thereby supporting improved therapeutic efficacy. The drug delivery systems exhibited an improved cytotoxicity profile compared to the free compounds while retaining their antioxidant activity, demonstrating the potential of the developed carriers for the production of highly effective delivery systems for natural bioactive compounds.

Perspectives for future research in the next 3 years:

- Development of procedures for the synthesis of silicate materials using "green" and economically viable approaches aimed at minimizing environmental impact and obtaining sustainable materials. For the implementation of this task, a variety of non-toxic, renewable, and cost-effective substances will be evaluated as structure-directing agents/templates. Surfactant molecules based on sugars, lipids, and biodegradable polymers will be investigated in reactions with conventional alkoxysilanes in order to assess their potential as suitable alternatives to widely used toxic structure-directing agents (quaternary ammonium salts and PEO-based block copolymers). Here, different approaches for template removal will also be explored. As an alternative to conventional high-temperature calcination, methods such as extraction with non-toxic solvents and low-temperature calcination will be employed. Furthermore, the possibility for regeneration, reuse, and recycling of the applied surfactants and solvents will be systematically examined. The procedures will be optimized with respect to efficiency, environmental sustainability, and economic feasibility.
- Applying innovative approaches for surface modification of the obtained materials with respect of their use as adsorbents for CO_2 capture and storage. To obtain efficient CO_2 adsorbents, various strategies—both post-synthetic and in situ (during synthesis)—will be employed for the surface modification of silicate carriers with organic functionalities containing primary, secondary, and cyclic amino groups. The modification conditions

(temperature, time, concentration of the modifying agent, etc.) will be systematically optimized in order to achieve materials with maximized adsorption performance.

- Synthesis of highly efficient adsorbents for the purification and isolation of biologically active compounds of natural origin. The efficiency of various approaches for the surface functionalization of porous silicates with selective groups will be systematically evaluated. Depending on the chemical nature of the target compounds, the silicates will be modified with metal species or organic functionalities, and the performance of the resulting adsorbents will be assessed in processes involving the isolation or separation of naturally derived compounds (e.g., flavonoids, proteins, enzymes) from complex matrices (e.g., extracts, fermentation products).

- Development of sustainable materials as platforms for biologically active substances in various pharmaceutical formulations. For the implementation of this task, methods for the synthesis of composite materials of the mesoporous silica–biodegradable polymer and mesoporous silica–lipid types will be developed. The preparation of these composites will employ porous silicates derived from waste biomass or synthesized using environmentally friendly templates and procedures. Within this framework, the synthesis protocols for the composite materials will be optimized with the aim of minimizing energy consumption and waste generation, and the use of non-toxic solvents under mild conditions (neutral pH, room temperature, etc.) will be systematically investigated.

- Preparation and submission of proposals to national and international competitive research funding programs.

- Collaborations with researchers from scientific institutes and higher education institutions in Bulgaria (Faculty of Pharmacy, Medical University of Sofia; Institute of Catalysis, Bulgarian Academy of Sciences; Institute of Inorganic Chemistry, Bulgarian Academy of Sciences; Faculty of Chemistry and Pharmacy, Sofia University) and abroad (National Institute of Chemistry, Slovenia; University of Namur, Belgium).

- Dissemination of research results through the participation at international scientific events and publication in peer-reviewed international journals.

References:

1. Trendafilova, I.; Szegedi, A.; Mihály, J.; Momekov, G.; Lihareva, N.; Popova, M. Preparation of Efficient Quercetin Delivery System on Zn-Modified Mesoporous SBA-15 Silica Carrier. *Mater. Sci. Eng. C* **2017**, *73*, doi:10.1016/j.msec.2016.12.063.
2. Trendafilova, I.; Chimshirova, R.; Momekova, D.; Petkov, H.; Koseva, N.; Petrova, P.; Popova, M. Curcumin and Capsaicin-Loaded Ag-Modified Mesoporous Silica Carriers: A New Alternative in Skin Treatment. *Nanomaterials* **2022**, *12*, doi:10.3390/nano12173075.
3. Popova, M.; Trendafilova, I.; Tsacheva, I.; Mitova, V.; Kyulavska, M.; Koseva, N.; Mihály, J.; Momekova, D.; Momekov, G.; Aleksandrov, H.A.; et al. Amino-Modified KIT-6 Mesoporous Silica/Polymer Composites for Quercetin Delivery: Experimental and Theoretical Approaches. *Microporous Mesoporous Mater.* **2018**, *270*, doi:10.1016/j.micromeso.2018.05.002.
4. Trendafilova, I.; Mihály, J.; Momekova, D.; Chimshirova, R.; Lazarova, H.; Momekov, G.; Popova, M. Antioxidant Activity and Modified Release Profiles of Morin and Hesperetin Flavonoids Loaded in Mg- or Ag-Modified SBA-16 Carriers. *Mater. Today Commun.* **2020**, *24*, 101198, doi:https://doi.org/10.1016/j.mtcomm.2020.101198.
5. Trendafilova, I.; Lazarova, H.; Chimshirova, R.; Trusheva, B.; Koseva, N.; Popova, M. Novel Kaempferol Delivery Systems Based on Mg-Containing MCM-41 Mesoporous Silicas. *J. Solid State Chem.* **2021**, *301*, 122323, doi:https://doi.org/10.1016/j.jssc.2021.122323.
6. Szegedi, Á.; Popova, M.; Trendafilova, I.; Trif, L.; Mihály, J.; Makk, J.; Mavrodinova, V. Bicomponent Drug Formulation for Simultaneous Release of Ag and Sulfadiazine Supported on Nanosized Zeolite Beta. *Nano-Structures & Nano-Objects* **2020**, *24*, 100562, doi:https://doi.org/10.1016/j.nanoso.2020.100562.
7. Popova, M.; Mihaylova, R.; Momekov, G.; Momekova, D.; Lazarova, H.; Trendafilova, I.; Mitova, V.; Koseva, N.; Mihályi, J.; Shestakova, P.; et al. Verapamil Delivery Systems on the Basis of Mesoporous ZSM-5/KIT-6 and ZSM-5/SBA-15 Polymer Nanocomposites as a Potential Tool to Overcome MDR in Cancer Cells. *Eur. J. Pharm. Biopharm.* **2019**, *142*, doi:10.1016/j.ejpb.2019.07.021.
8. Szegedi, Á.; Trendafilova, I.; Mihály, J.; Lázár, K.; Németh, P.; Momekov, G.; Momekova, D.; Marinov, L.; Nikolova, I.; Popova, M. New Insight on Prednisolone Polymorphs in Mesoporous Silica/Maghemite Nanocomposites. *J. Drug Deliv. Sci.*

Technol. **2020**, *60*, doi:10.1016/j.jddst.2020.102092.

9. Popova, M.; Koseva, N.; Trendafilova, I.; Lazarova, H.; Mitova, V.; Mihály, J.; Momekova, D.; Momekov, G.; Koleva, I.Z.; Aleksandrov, H.A.; et al. Tamoxifen Delivery System Based on PEGylated Magnetic MCM-41 Silica. *Molecules* **2020**, *25*, doi:10.3390/molecules25215129.
10. Szegedi, Á.; Shestakova, P.; Trendafilova, I.; Mihayi, J.; Tsacheva, I.; Mitova, V.; Kyulavska, M.; Koseva, N.; Momekova, D.; Konstantinov, S.; et al. Modified Mesoporous Silica Nanoparticles Coated by Polymer Complex as Novel Curcumin Delivery Carriers. *J. Drug Deliv. Sci. Technol.* **2019**, *49*, doi:10.1016/j.jddst.2018.12.016.

LIST OF SCIENTIFIC PUBLICATIONS
submitted under indicator "B" for the competition for the academic position of Associate Professor

Publications in Q1 journals:

1) Trendafilova,I., Szegedi,A., Mihály,J., Momekov,G., Lihareva,N., Popova,M.. *Preparation of efficient quercetin delivery system on Zn-modified mesoporous SBA-15 silica carrier*. Materials Science and Engineering C, 73, Elsevier, **2017**, 285-292, https://doi.org/10.1016/j.msec.2016.12.063

2) Popova, M., Trendafilova, I., Tsacheva, I., Mitova, V., Kyulavska, M., Koseva, N., Mihály, J., Momekova, D., Momekov, G., Aleksandrov, H.A., Marinova, S.G., Petkov, P.S., Vayssilov, G.N., Szegedi, A.. *Amino-modified KIT-6 mesoporous silica/polymer composites for quercetin delivery: Experimental and theoretical approaches*. Microporous and Mesoporous Materials, 270, **2018**, 40-47, https://doi.org/10.1016/j.micromeso.2018.05.002

3) Trendafilova, I., Chimshirova, R., Momekova, D., Petkov, H., Koseva, N., Petrova, P., Popova, M. *Curcumin and Capsaicin-Loaded Ag-Modified Mesoporous Silica Carriers: A New Alternative in Skin Treatment*. Nanomaterials, 12, 17, **2022**, 2079-4991, https://doi.org/10.3390/nano12173075

Publications in Q2 journals:

4) Trendafilova, I., Mihaly, J., Momekova, D., Chimshirova, R., Lazarova, H., Momekov, G., Popova, M.. *Antioxidant activity and modified release profiles of morin and hesperetin flavonoids loaded in Mg- or Ag-modified SBA-16 carriers*. Materials Today Communications, 24, **2020**, 10119, https://doi.org/10.1016/j.mtcomm.2020.101198

5) Trendafilova, I., Lazarova, H., Chimshirova, R., Trusheva, B., Koseva, N., Popova, M.. *Novel kaempferol delivery systems based on Mg-containing MCM-41 mesoporous silicas*. Journal of Solid State Chemistry, 301, **2021**, 122323, https://doi.org/10.1016/j.jssc.2021.122323

LIST OF SCIENTIFIC PUBLICATIONS

submitted under indicator " Г", which do not repeat those submitted in other competitions for holding academic positions and acquiring scientific degrees

Publications in Q1 journals:

6) Szegedi, A., Popova, M., Trendafilova, I., Trif, L., Mihály, J., Makk, J., Mavrodinova, V.. *Bicomponent drug formulation for simultaneous release of Ag and sulfadiazine supported on nanosized zeolite Beta*. Nano-Structures & Nano-Objects, 24, **2020,** 100562, https://doi.org/10.1016/j.nanoso.2020.100562

7) Popova,M., Mihaylova,R., Momekov,G., Momekova,D., Lazarova, H, Trendafilova,I., Mitova,V., Koseva,N., Mihályi,J., Shestakova,P., St. Petkov,P., Aleksandrov,H.A., Vayssilov,Georgi N., Konstantinov,S., Szegedi,Á.. *Verapamil delivery systems on the basis of mesoporous ZSM-5/KIT-6 and ZSM-5/SBA-15 polymer nanocomposites as a potential tool to overcome MDR in cancer cells*. European Journal of Pharmaceutics and Biopharmaceutics, 142, **2019**, 460-472, https://doi.org/10.1016/j.ejpb.2019.07.021

8) Szegedi, A., Trendafilova, I., Mihály, J., Lázár, K., Németh, P., Momekov, G., Momekova, D., Marinov, L., Nikolova, I., Popova, M.. *New insight on prednisolone polymorphs in mesoporous silica/maghemite nanocomposites*. Journal of Drug Delivery Science and Technology, 60, **2020**, 102092, https://doi.org/10.1016/j.jddst.2020.102092

9) Popova, M., Koseva, N., Trendafilova, I., Lazarova, H., Mitova, V., Mihály, J., Momekova, D., Momekov, G., Koleva, I., Aleksandrov, H., Vayssilov, G., Szegedi , A.. *Tamoxifen Delivery System Based on PEGylated Magnetic MCM-41 Silica*. Molecules, 25, 21, **2020**, 5129, https://doi.org/10.3390/molecules25215129

10) Popova, M., Koseva, N., Trendafilova, I., Lazarova, H., Mitova, V., Mihály, J., Momekova, D., Konstantinov, S., Koleva, I., Petkov, P., Vayssilov, G., Aleksandrov, H., Szegedi, A.. *Design of PEG-modified magnetic nanoporous silica based miltefosine delivery system: Experimental and theoretical approaches*. Microporous and Mesoporous Materials, 310, **2021**, 110664, https://doi.org/10.1016/j.micromeso.2020.110664

11) Grozdanova, S., Trendafilova, I., Szegedi, A., Shestakova, P., Mitrev, Y., Slavchev, I., Simeonov, S., Popova, M.. Mesoporous Silica Xerogels Prepared by p-toluenesulfonic

AcidAssisted Synthesis: Piperazine-Modification and CO2 Adsorption. Nanomaterials, 15, 19, **2025**, 20794991, https://doi.org/10.3390/nano15191459

12) Trendafilova, I., Popova, M.. *Porous Silica Nanomaterials as Carriers of Biologically Active Natural Polyphenols: Effect of Structure and Surface Modification*. Pharmaceutics, 16, 8, **2024,** 1004, https://doi.org/10.3390/pharmaceutics16081004

Publications in Q2 journals:

13) Szegedi, A., Shestakova, P., Trendafilova, I., Mihayi, J., Tsacheva, I., Mitova, V., Kyulavska, M., Koseva, N., Momekova, D., Konstantinov, S., Aleksandrov, H. A., Petkov, P. St., Koleva, I. Z., Vayssilov, G. N., Popova, M.. *Modified mesoporous silica nanoparticles coated by polymer complex as novel curcumin delivery carriers*. Journal of Drug Delivery Science and Technology, 49, Elsevier, **2019**, 700-712, https://doi.org/10.1016/j.jddst.2018.12.016

Publications in Q3 journals:

14) Trendafilova, I., Momekova, D., Koseva, N., Popova, M.. *Magnetic porous silica-lipid bilayer hybrid carriers for target delivery of curcumin*. Comptes rendus de l'Acad´emie bulgare des Sciences, 75, 10, **2022**, 1437, https://doi.org/10.7546/CRABS.2022.10.05

Publications in Q4 journals:

15) Trendafilova, I., Popova, M., Momekova, D., Szegedi, A., Momekov, G., Zgureva, D., Boycheva, S.. *Silver and quercetin loaded nanostructured silica materials as potential dermal formulations*. Bulgarian Chemical Communications, 49, **2017**, 51-58, https://www.bcc.bas.bg/BCC_Volumes/Volume_49_Special_F_2017/BCC-F-IT-51-58.pdf

16) Popova, M., Trendafilova, I., Tsacheva, I., Georgieva, N., Koseva, N., Szegedi, A., Mihály, J., Novak-Tusar, N.. *Preparation of quercetin delivery systems on the basis of amino-modified KIL-2 mesoporous silica*. Bulgarian Chemical Communications, 50, Special Issue C, **2018**, 190-194, https://bcc.bas.bg/BCC_Volumes/Volume_50_Special_C_2018/pdf/BCC-50-C2018-190-194-Popova-54.pdf