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EUROPEAN REGIONAL
DEVELOPMENT FUND

OPERATIONAL PROGRAMME
SCIENCE AND EDUCATION
FOR SMART GROWTH

Project № BG05M2OP001-1.002-0005, Personalized Innovative Medicine (PERIMED)

Term of implementation: 30 March 2018 – 31 December 2023
Starting funding: 23 472 019.71 leva (11 982 673 Euro)

Leading organization:

Medical University, (MU Plovdiv). Plovdiv, Bulgaria, https://mu-plovdiv.bg

Partners:

University of Plovdiv "Paisii Hilendarski", (PU) Plovdiv, Bulgaria, www.uni-plovdiv.bg_

Institute of Mineralogy and Crystallography "Acad. Ivan Kostov", Bulgarian Academy of Sciences (IMC-BAS), Sofia, Bulgaria, http://www.imc.bas.bg

Associated partners:

University Multi-profile Hospital for Active Treatment /UMHAT/ "SAINT GEORGE"

NEOPHARM BULGARIA Ltd., Sofia, Bulgaria, http://neopharm.bg/

University of Manchester, UK

Instituto De Agroquimica y Tecnologia de Alimentos as a part of the State Agency, Madrid, Spain

This Special issue of the Bulgarian Chemical Communications contains selected papers, reported as oral or poster presentations at the final Conferences organized by the partners of the Center of Competence in Personalized Innovative Medicine.

The Center of Competence in Personalized Innovative Medicine (PERIMED) stands as a pivotal entity in the transforming of biomedical research into viable therapies, significantly improving the quality of life for patients and the general population. Ini-

tially, it brings together a consortium of three partners: the Medical University of Plovdiv, Plovdiv University "Paisii Hilendarski", and the Institute of Mineralogy and Crystallography at the Bulgarian Academy of Sciences. This collaborative effort lays the grounds for advancements in personalized medicine.

With an eye towards future developments, PERIMED is ready for strategic expansion in its next phase, PERIMED II. This expansion includes broadening the consortium to include four partners by incorporating an NGO. This new addition is tasked with a critical role in bridging the gap between PERIMED research and business. It will offer initiatives in managing interactions between research entities and businesses, overseeing the development and guidance of spin-off and startup companies, and facilitating the transfer of intellectual properties. This evolution marks a significant step in enhancing the consortium capacity to translate scientific achievements into commercial opportunities, fostering a more direct impact on both the healthcare industry and society at large.

The Center of Competence PERIMED is dedicated to fostering collaborative research and innovation among scientists from the Medical University of Plovdiv, Plovdiv University "Paisii Hilendarski", and the Institute of Mineralogy and Crystallography at the Bulgarian Academy of Sciences. Its goal is to augment the market relevance of their research and development endeavors, thereby facilitating business partnerships. The project bridges strategic commitment to innovation, collaboration, and the practical application of research findings, ensuring the project's sustained influence on personalized medicine and its accessibility to the market.

The establishment of PERIMED as a nationally significant scientific infrastructure complex, integrated into European networks in personalized medicine and focused on three primary areas:

- *Molecular and biological methodologies for application in personalized medicine and implementation of a personalized approach in the treatment of critically ill patients;*
- *Innovative drug delivery systems for targeted therapy and personalized medicine;*
- *Bioengineering technologies and biosensors.*

The strategy to achieve the project's objectives was built on three pillars:

- *Providing state-of-the-art equipment;*
- *Developing advanced health-related technologies; and*
- *Building academic resources with significant potential and scientific capacity.*

The successful implementation and advancement of the PERIMED project is fully achievable, as all participants recognize the profound responsibility they bear towards human health. The project's execution is expected to offer substantial benefits to society, categorized into health-related, educational, and economic aspects:

Health related:

- Enhanced quality of diagnostic and therapeutic practices based on contemporary scientific principles;
- Adoption of personalized treatment approaches for cancer patients and critically ill individuals;
- Tailored use of medical products to fit patient-specific needs;
- Increased effectiveness and safety of therapeutic methods;
- Improved living conditions, leading to more patients being cured and saved.

Educational:

- Acquisition of new knowledge vital for scientific and entrepreneurial ventures;
- Development in various pharmaceutical science and biotechnology domains – including nanotechnology, alternative drug administration routes, targeted therapy, intensive medicine, immobilized biocatalysts, natural bioactive substances, biopolymers, new materials, and biosensors;
- Enhancement of capacity for innovative scientific research;
- Conducting research to European standards;
- Encouraging young scientists to pursue their careers in Bulgaria.

Economic:

- Business growth through the application of innovative scientific findings and the commercialization of technological innovations;
- Reduced treatment costs for socially significant diseases;
- Creation of a new model for research-business interaction, fostering mutual benefits;
- Job creation;
- Enhanced collaboration among professionals in medicine, genetics, pharmacy, bioinformatics, medical statistics, and pharmacoeconomics;

- Improved management of oncology and intensive care units;
- Establishment of new spin-off and startup companies with the potential for business and profit.

From March 30, 2018, to December 31, 2023, the PERIMED consortium utilized the funding of 23,472,019.71 leva, dedicating at least 75% to infrastructure and the remainder to maintenance, specializations, and dissemination, among other necessities. The consortium faced significant administrative burdens at the project's onset due to evolving implementation rules set by the Executive Agency "Programme Education" and the impact of the COVID-19 pandemic, which basically halted research activities and reduced the Medical University of Plovdiv's administrative capacity (March 2020 to March 2022). To achieve its objectives, PERIMED worked on the execution of twelve work packages (WP), ranging from the creation and validation of gene panels for cancer diagnosis to the development of drug delivery systems and biosensors, showcasing a comprehensive approach to advancing personalized medicine.

WP 1. Creation and validation of a panel of genes for monitoring of tumor heterogeneity, molecular resistance, tumor load and minimum residual disease in patients with verified breast cancer;
WP 2: Creation and validation of a panel of genes for precise molecular-genetic diagnosis in patients with chronic myeloid leukemia (CML) and monitoring of minimal residual disease;
WP 3. Molecular biomarkers for medical application;
WP 4. Application and development of the method of flow cytometric monitoring of the minimal residual disease in children with acute lymphoblastic leukemia;
WP 5. Creation and introduction of comprehensive personalized approach in critically ill patients;
WP 6. Molecular biomarkers of the microbiota of the gastrointestinal tract;
WP 7. Immuno-biomarkers for tumor and autoimmune diseases;
WP 8. Drug-delivery systems for targeted effect of medications and personalized medicine;
WP 9: physicochemical characterization of innovative medical forms;
WP10. Biocatalysts and natural bioactive substances;
WP 11. Biopolymers and new materials;
WP 12. Biosensors.

This main part of the investment led to the establishment of modern research laboratories by the consortium partners, each specialized in crucial areas of personalized medicine. This infrastructure was instrumental in advancing research and development efforts. The laboratories and acquired state-of-the-art equipment supported the implementation of twelve work packages:

Medical University – Plovdiv, PERIMED laboratories:

1. Drug delivery systems for targeted effect of drugs and personalized medicine,
2. Molecular genetic markers for medical use.

Plovdiv University "Paisii Hilendarski", PERIMED laboratories:

3. Molecular biomarkers of the microbiota;
4. Immunobiomarkers;
5. Biocatalysis and biologically active substances;
6. Biopolymers and new materials;
7. Biosensors.

Institute of Mineralogy and Crystallography, BAS, PERIMED laboratory:

8. Laboratory for physico-chemical control of innovative medicinal forms (pharmaceutical ingredients)

The laboratory of "Drug delivery systems for targeted effect of drugs and personalized medicine", has been equipped with a nano-spray dryer, microencapsulation equipment, and highly specialized equipment for structural-morphological characterization of drug delivery systems and for studying biological samples. Namely, a state-of the-art FEI 200 cryo transmission electron microscope (cryoTEM) for working at low temperatures and a scanning electron microscope (Figure 1). The laboratory also has four flow cell apparatus necessary for the biopharmaceutical and pharmacokinetic characterization of the developed drug carriers, to ensure accuracy, repeatability, and a high degree of correlation between *in vitro/in vivo* studies. There is also suitable equipment for cell culturing, allowing for tests on drug toxicity and biocompatibility and achieving targeted drug release in experimental animals.

The laboratory of "Molecular genetic markers for medical use" supports WPs 1, 2 and 3. It is fo-

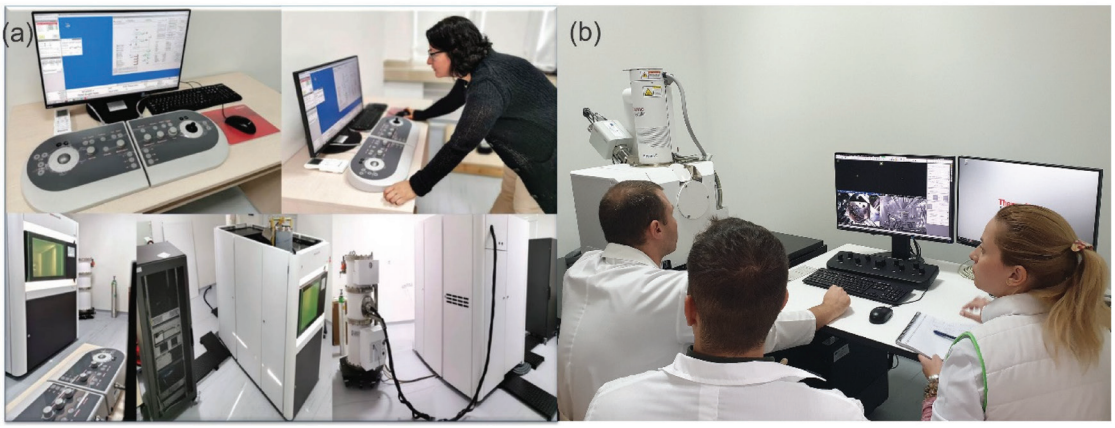

Figure 1. Installed (a) FEI 200 cryo-transmission electron microscope (cryoTEM) and (b) scanning electron microscope at the laboratory of Drug delivery systems for targeted effect of drugs and personalized medicine – MU Plovdiv.

cused on establishing and use of new molecular biomarkers for medical use and diagnosis of breast cancer (WP 1) and chronic myeloid leukemia (CML) and minimal residual disease (WP 2), while WP 3 combines the results of 1 and 2 and thus improves the prognosis of treatment of the diseases studied and monitored. The major acquired infrastructure includes a next generation sequencer and a digital droplet PCR (Figure 2). In brief, a protocol has been developed to detect variants of a specific (DPYD) gene – the cause of severe side effects in the treatment of cancer patients, the development and validation of a panel of genes for accurate molecular diagnosis in patients with CML is being tested and tests for detection of target mutations associated with resistance to antitumor treatment by digital droplet PCR are being developed.

The application and development of the method of flow cytometric monitoring of the minimum residual disease (MRD) in children with acute lymphoblastic leukemia (ALL) required the acquisition of a Cell Sorter based on 4 laser multiparameter flow cytometry (Figure 3). As result of WP4 were created and validated:

Figure 2. Major infrastructure and equipment at the laboratory "Molecular genetic markers for medical use" (a) next generation sequencer and (b) digital droplet PCR system.

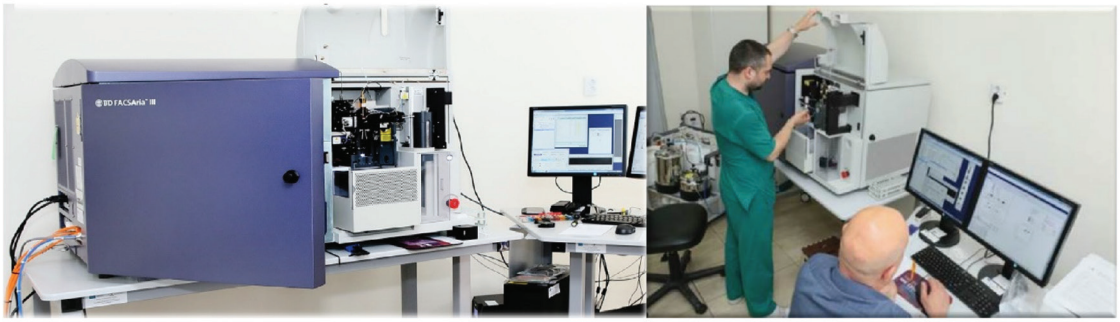

Figure 3. Cell Sorter based on 4 laser multiparameter flow cytometry.

1. A modern methodology for the study of MRD in children with hematological and oncological diseases;

2. An innovative science-based algorithm for research and assessment of the minimum residual disease in children with ALL;

3. Standardized panels for the study of MRB implemented as an objective criterion for monitoring the effect of the treatment.

Work package 5 was focused on the development of a Critically ill patient monitoring system (Web based Portal for telemonitoring):

- through the simultaneous tracking and analysis of multiple patient's vital parameters;
- detection and objectification of their relationships (Algorithm for personalized assessment and follow-up).

Work packages 6 and 8 worked together on Nano- and microcarriers of drugs with different polymers for targeted release in the gastrointestinal tract (spray drying, ionotropic gelling):

- Optimal condition analyzes for the highest yield of carriers, high productivity, reproducibility of the results without loss of material;
- Protecting the drug from the aggressive stomach environment – the drug reaching the target unchanged.

Similarly, Work packages 8 and 9 worked on the development of new inorganic drug carriers for antitumor therapy:

- Four types of silicate micro- or nano-carriers were developed by WP 9 (Figure 4b);
- The processes of activation and loading of the silicate nanocarriers with paclitaxel and other drugs, used in antitumor therapy were optimized (WP 9);
- The release of paclitaxel has been studied by the liquid chromatographic method with mass detection WP 8.

In addition WP 8 developed models of casein nanoparticles (Figure 4a) with daunorubicin as promising drug carriers to achieve highly effective and safe antitumor therapy. Another study explored the use of casein micelles as nanocarriers for benzydamine delivery, demonstrating the formulation of nanoparticles for controlled and targeted drug release. This aligns with PERIMED's goal to develop innovative drug delivery systems, showcasing the potential of naturally occurring materials in improving therapeutic outcomes (https://www.mdpi.com/2073-4360/13/24/4357).

The next five laboratories are located in the Centre of technologies of the Paisii Hilendarski University of Plovdiv – an infrastructure, building, that has been a subject to construction work, funded by the PERIMED project (Figure 5). The laboratories supported the research of five WPs:

- WP 6. Molecular biomarkers of the microbiota of the gastrointestinal tract (Figure 6);
- WP 7. Immuno-biomarkers for tumor and autoimmune diseases;
- WP 10. Biocatalysts and natural bioactive substances (Figure 7);

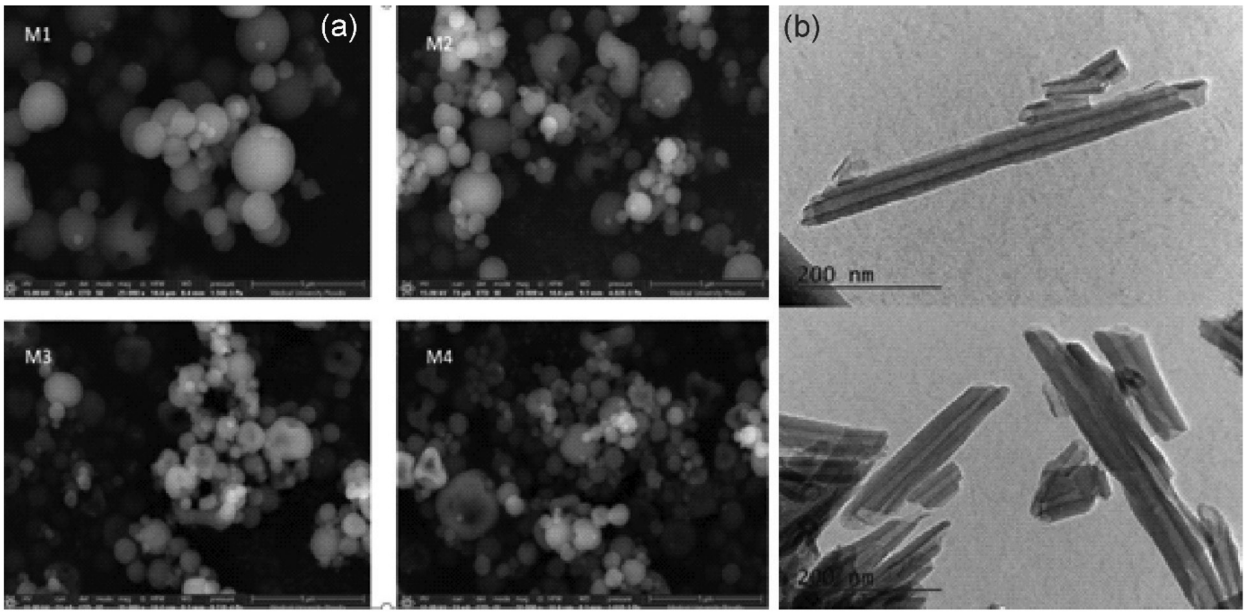

Figure 4. SEM visualization of the polymer nanoparticles (a) and (b)TEM visualization of the silicate nanocarriers.

Figure 5. The Centre of technologies at the Paisii Hilendarski University of Plovdiv.

Figure 6. Laboratory of Molecular biomarkers of the microbiota of the gastrointestinal tract.

Figure 7. Laboratory of Biocatalysts and natural bioactive substances.

- WP 11. Biopolymers and new materials (Figure 8);
- WP 12 Biosensors (Figure 9).

As a result of the work, the development of a biosensor for detecting dopamine and L-epinephrine confirms PERIMED's work on bioengineering technologies. This biosensor, based on laccase-catalyzed assays and immobilized on a gold-modified electrode, represents an innovative approach to neurotransmitter measurement, with potential applications in both clinical diagnostics and research (https://www.mdpi.com/2079-6374/12/9/719).

Figure 8. Laboratory of Biopolymers and new materials.

Figure 9. Laboratory of Biosensors.

The "Laboratory for physico-chemical control of innovative medicinal forms" (pharmaceutical ingredients) is located at the Institute of Mineralogy and Crystallography, BAS. The Infrastructure premises of the laboratory were subject to complete renovation according to the requirements of the equipment. The Laboratory supports the research work of WPs 8 to 12, while the main effort is concentrated on the physico-chemical characterization and control of existing an innovative medicinal forms, active pharmaceutical ingredients and excipients. The main laboratory equipment includes a diffractometer (Empyrean, Figure 10), DSC (TA-250, Figure 11), surface area and porosity analyzer (BETsurface, micro- and meso-pore size and porosity) (3FLEX, Figure 13), Isothermal titration calorimetry – ICT TA-Affinity (Figure 12), UV-Vis Carry 4000 and planetary ball mill Pulverisette 7 premium Fritsch. Additional infrastructure allowing synthesis, antibacterial, elemental analysis etc., to the support of the research is also available. New and existing pharmaceutical entities (active pharmaceutical ingredient-APIs) are required to possess physicochemical characteristics that result in adequate reproducibility. The most common problem is the low solubility of the pharmaceutical entities that is very often overcome by the engineering of metastable phases. Physicochemical characterization focuses on the determination of thermal stability, polymorphism, dissolution or hydration, and the presence of undesirable contaminants in pharmaceutical products. The package is focused on providing data and investigation of solid form (polymorphs) by XRD, thermal stability by DSC, loading of active substances and their interaction with excipients (auxiliary substances) by surface area, strong interaction by ITC.

In pharmaceutical research, the focus of WP 9 was on developing drug delivery systems using synthetic zeolites. This involved synthesizing zeolite phases, loading drugs onto them, and studying drug release. Different surface modifications and methods were explored. Dexamethasone was initially tested, and later Taxol and Cyclophosphamide were added for analysis. UV-Vis studies were conducted, leading to several outcomes:

- A methodology for loading paclitaxel onto HNT1 was established, including specific activation and pH-dependent release;
- Another porous material for protected drug release in heart conditions is being investigated.

The applied research was focused on the polymorphism that is a significant problem for pharma-

Figure 10. Diffractometer Empyrean. Applications: qualitative phase/polymorphs and quantitative analysis (wt%), Cu (1.5406 Å) and Co (1.7902 Å) sources; modes: reflection, transmission, microdiffraction (spot of 50 µm), measurements at different temperatures and different gas environments, low temperature (to –200 °C), high-temperature (to +600 °C), controlled atmosphere, SAXS/USAX, thin layers (up to 500 nm), microdiffraction, xyz stage etc.

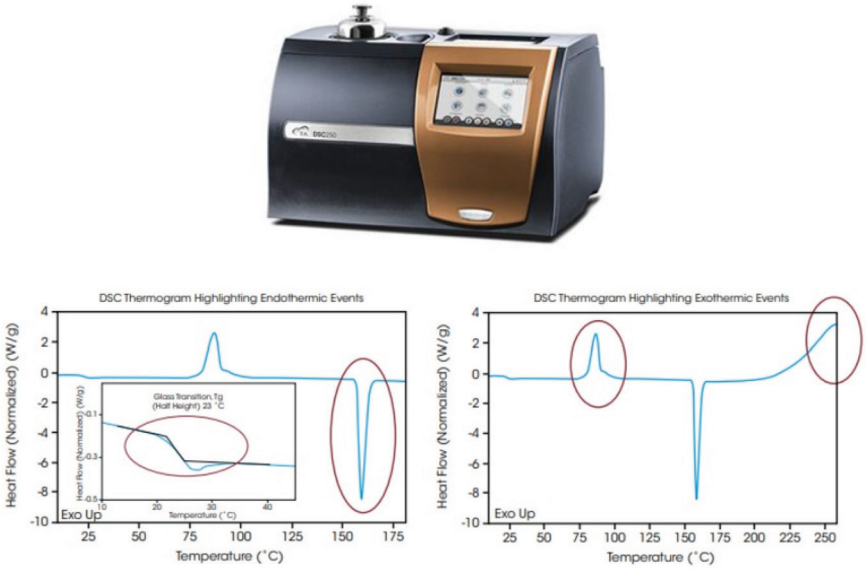

Figure 11. DSC250 TA Instruments: melting points, phase transitions, solvent evaporation, dehydration and rehydration, analysis of purity ASTM. Small amount of sample 0.1 mg, ambient, nitrogen, argon conditions. –120 to 450 °C, suitable for Protein/DNA denaturation, folding analyses.

ceutical industry. A consolidated analytical technique based on Powder X-ray Diffraction has been developed, being the definitive test for the identification of polymorphs and crystal phases. However, its application for quantitative analysis is hindered by matrix effects: the presence of excipients requires a complete knowledge of samples' composition. Thus, univariate calibration methods require the matrix effect to be studied and adjusted but still suffer from the co-presence of different phases in the sample. Multivariate analysis is the only way to bypass problems. In particular, the

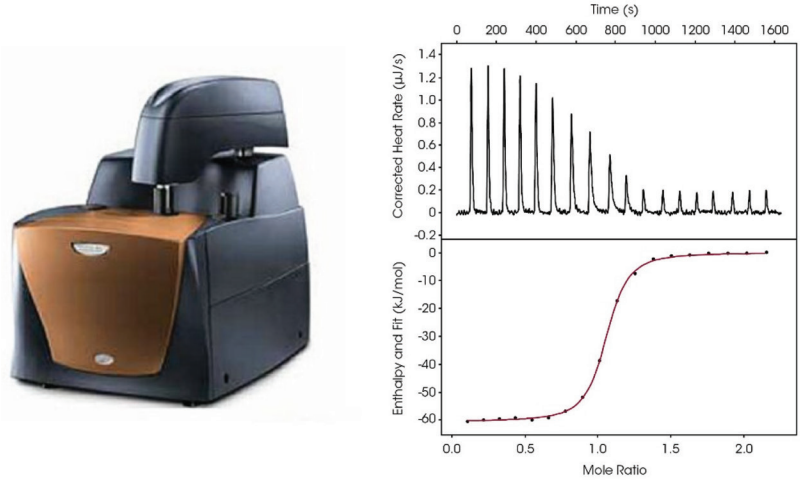

Figure 12. Affinity ITC, TA Instruments – detects interaction between protein/DNA/antibody and drug (ligand), works with solutions, cell volume (190 µl), syringe volume (250 µl), temperature range (2–80 °C), measured heat range (0.04–5000 µJ).

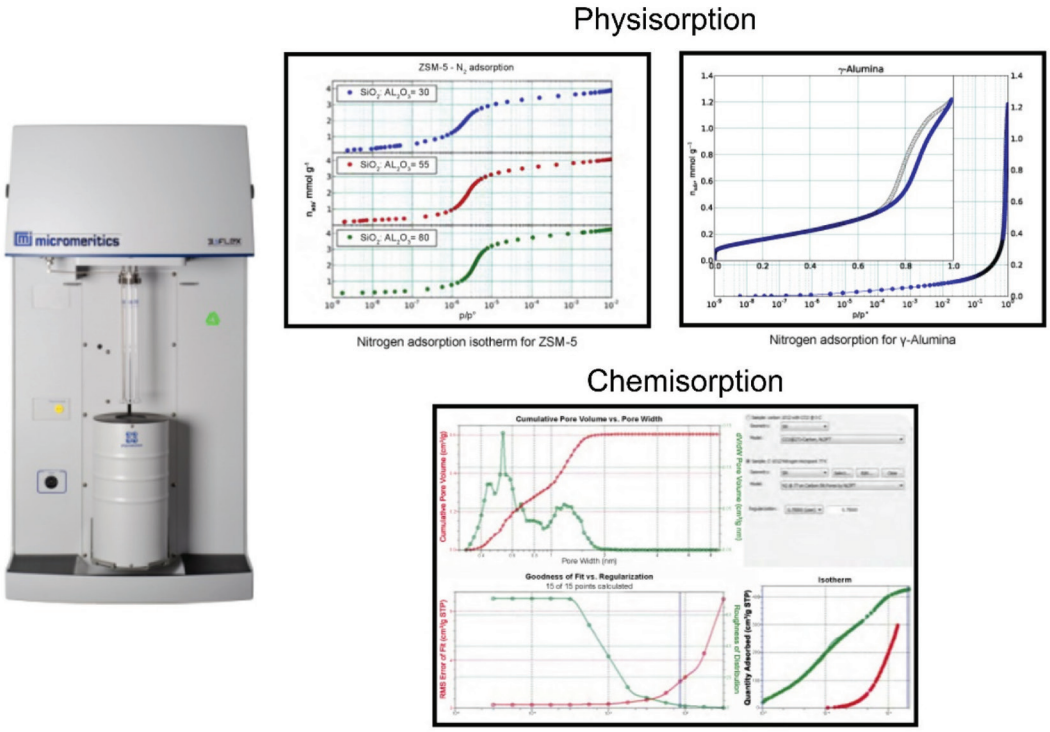

Figure 13. Surface area analyzer – BET, 3Flex Micromeritics: nanopowders surface area, detects the loading of drugs on nanocarriers, determines suface are, pores sizes.

multivariate standard addition method (SAM) is promising. Combing PXRD with complimentary DSC/TGA etc. will provide a cross-check for definite polymorph analysis.

The main outcomes of PERIMED project were presented at the conferences organized by the three partners "Personalized Innovative Medicine" (PERIMED) 4-th and 5-th MU-Plovdiv, 5-th and 6-th December 2023 Plovdiv, Bulgaria, IMC–BAS.

Through its dedicated work packages, PERIMED has carried out pioneering research in personalized medicine. Notable scientific achievements include

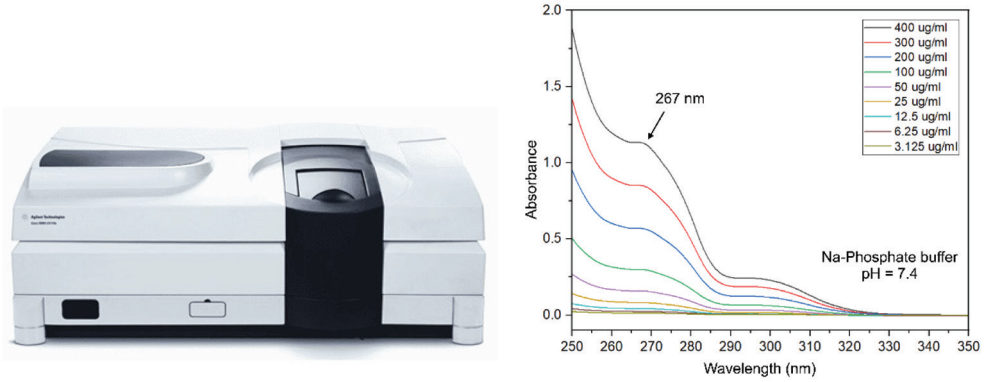

Figure 14. UV-VIS Cary 4000 , range 175–900 nm; works with solid and liquid sample solid volume ~1 cm^3, liquid sample volume from 0.2 to 3.5 ml; the system is optimal for analyzing solid samples or liquid biological samples with minimal sample preparation. Monitoring drug release, degradation, sedimentation, comparing solutions *vs* solid state in APIs.

Figure 15. Extract of the Conference program.

the creation and validation of gene panels for cancer diagnosis and monitoring, the development of novel drug delivery systems for targeted therapy, and the advancement of bioengineering technologies. These efforts have led to improved diagnostic and therapeutic practices, tailoring treatments to individual patient needs.

PERIMED has successfully established eight modern research laboratories across its consortium partners, equipped with cutting-edge technology. These laboratories specialize in various critical areas, including drug delivery systems, molecular genetic markers, molecular biomarkers, immunobiomarkers, biocatalysis, biopolymers, new materials, and biosensors. This infrastructure and the studies, funded and facilitated by the PERIMED project, reflect the comprehensive effort to advance personalized medicine through cutting-edge research in pharmacogenetics, drug delivery systems, immunology, and biosensor technology. Each effort contributes to the overarching goal of enhancing patient care by tailoring medical treatments to individual genetic profiles and physiological needs, paving the way for more precise and effective therapies.

PERIMED's work has directly benefited society across health-related, educational, and economic dimensions. It has contributed to enhanced quality of life through improved diagnostic and therapeutic methodologies, personalized treatment approaches, and increased effectiveness and safety of medical treatments. Additionally, PERIMED has played a pivotal role in education by providing new knowledge, encouraging young scientists, and enhancing research capacity.

The project has stimulated economic growth by promoting business development through innovative scientific findings. It has created new jobs, reduced diagnostics and treatment costs for significant diseases, and with the strategic inclusion of NGO in PERIMED II has expanded the consortium, enhancing its capability to bridge research and business. The founding of new spin-off and startup companies has also contributed to the economic landscape.

Throughout the 2018–2023 period, PERIMED's achievements have not only advanced the field of personalized medicine but also set a foundation for future innovations and collaborations. The consortium's dedication to transforming biomedical research into practical therapies has positioned it as a leader in personalized medicine, with a lasting impact on patient care, scientific research, and economic development.

Prof. Boris Shivachev,
Member of the Scientific board of PERIMED
Organizing committee of the final Conference "Personalized Innovative Medicine" (PERIMED), December 2023, Plovdiv, Bulgaria