

**"VICTOR BABEȘ" UNIVERSITY OF
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**COMPLEX NETWORKS AS AN INTEGRATIVE
FRAMEWORK IN SYSTEMS PHARMACOLOGY:
DRUG REPOSITIONING AND CLINICAL
IMPLICATIONS**

ABSTRACT

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The habilitation thesis entitled *"Complex Networks as an Integrative Framework in Systems Pharmacology: Drug Repositioning and Clinical Implications"* summarizes the personal scientific, academic, and professional achievements in the postdoctoral period 2015-2021, as well as the main directions of development in research and academic areas. Our scientific papers have contributed to (i) optimizing the biopharmaceutical properties of drugs through inclusion complexes with cyclodextrins, (ii) modeling the host-guest molecular interaction (i.e., drug-cyclodextrin) and drug-target, and (iii) application of complex networks in the pharmaceutical sciences (for drug repositioning, by analyzing drug-drug interactions and drug-target complex networks) and medical sciences (for phenotyping, prediction of severity and response to CPAP treatment of apnea patients).

The first chapter presents the scientific achievements. The first subchapter states the research motivation and the most important published papers (ISI articles, book chapters, in extenso and abstract papers published at conferences) and participation in scientific research projects. According to Clarivate Web of Science Core Collection (April 2021), our scientific activity has an impact characterized by a Hirsch index of 8, a total of 129 ISI citations (95 without self-citations).

As the principal author, we published 10 ISI scientific papers, with an impact factor between 0.538 and 4,259, of which four in the Q1 zone and 2 in the Q2 zone, with a cumulative impact factor of 21.994. As a co-author, we published 10 ISI papers with an impact factor between 0.538 and 2.776, of which 2 in the Q2 zone. We were also involved in 4 research projects: two international projects, a national project, and an internal project of the "Victor Babeș" University of Medicine and Pharmacy Timișoara. Of these, two projects are in progress; we are director of the experimental demonstrative project "Complexity science for precision pharmacy: predicting relevant drug interactions using complex network analysis" – HYPERION, won in the UEFISCDI competition PN-III-P2-2.1-PED-2019 (November 2020 – October 2022), and assistant manager and researcher in the project "Revolution of sleep diagnostics and personalized health care based on digital diagnostics and therapeutics with health data integration" - SLEEP REVOLUTION, ID 965417, in the European competition HORIZON 2020 (March 2021 – February 2025). We were also a researcher in *"Morpheus: a screening and monitoring system for sleep apnea syndrome,"* a LINDE Healthcare REAL Fund Project nr. 11289/ 14.10.2014 (2014-2016) and in *"The synthesis of new compounds with potential anticancer activity by means of chemical derivatisation and their characterization through hyphenated technique. The evaluation of the antiangiogenic, antitumor and anti-inflammatory activity"* – SYNTANTITUM, a research grant funded by UMFVBT in the III-C1-PCFI-2014/2015 competition.

The following subchapter presents the methods we applied to improve the biopharmaceutical properties of some angiotensin I conversion enzyme inhibitor drugs – namely, fosinopril and zofenopril – by forming inclusion complexes with cyclodextrins. Many new drug molecules have a poor solubility profile leading to a low bioavailability.

This drug category's "recovery" method is the formation of inclusion complexes with biocompatible cavitary macromolecules, particularly cyclodextrins. Cyclodextrins' structural features (a hydrophobic cavity able to accommodate lipophilic guest molecules and a hydrophilic outer surface) make them vectors of increasing solubility for many drugs in the biological environment; thus, they contribute to the increasing of oral bioavailability and reducing individual variability of this biopharmaceutical parameter. Preparing inclusion complexes of these two drugs with cyclodextrins is reasonable because this process increases their water solubility. Indeed, for fosinopril, this process improves the oral bioavailability and increases the stability in the presence of excipients; for zofenopril, it avoids oral absorption variability, increases the stability to oxidation that causes premature decomposition, and reduces the pharmacological effect. We analyzed the physicochemical properties of pure substances and their binary systems (namely, the physical mixture and the complex prepared by the kneading method) with specific instrumental techniques, such as UV spectrophotometry, FTIR/ATR-FTIR, powder X-ray diffraction, thermal analysis, in order to highlight the emergence of a new phase represented by the inclusion complex. The experimental results proved the formation of actual inclusion complexes between fosinopril and β -cyclodextrin, on the one hand, and zofenopril and two β -cyclodextrin derivatives (*i.e.*, 2-hydroxypropyl β -cyclodextrin and randomly methylated β -cyclodextrin), on the other hand. The fosinopril- β -cyclodextrin molecular docking confirms the FTIR results about forming an actual inclusion complex between the two components. The kneading method applied for the preparation of all these complexes is a simple technique that considerably increases the included drugs' water solubility, allowing for the manufacturing of these complexes in oral pharmaceutical dosage forms to overcome the drawbacks.

The third subchapter addresses applying the new science of complex networks in pharmacy, pharmacology, and medicine. We used the complex network tools to build a bipartite graph, *i.e.*, containing two categories of nodes – namely, nodes corresponding to cyclodextrins (13 cyclodextrins) and nodes corresponding to the included drugs (190 guest drugs), respectively. An edge links two nodes when a cyclodextrin includes a drug; the network comprises 367 links. The degree distribution analysis reveals that the complex network is scale-free, the distribution being of power-law type. These results show a specific pattern of guest drug-cyclodextrin relationship, which may be correlated with these guest substances' structure. This correlation can help predict whether or not a cyclodextrin includes a drug for which no pieces of evidence are available from experiments. Another approach is to build an inclusome, namely a complex network that characterizes drugs' properties included within cyclodextrins' cavity. The inclusome is a bipartite graph, in which the edges connect a cyclodextrin to a guest when they form an inclusion complex characterized by an optimal value for the parameter $K_{1:1}$ ($200 \text{ M}^{-1} \leq K_{1:1} \leq 5000 \text{ M}^{-1}$). In this network, β -cyclodextrin and 2-hydroxypropyl β -cyclodextrin are hubs, followed by γ -, α - and methyl β -cyclodextrin, which confirms the order of importance

of their use in pharmaceutical practice. The monopartite approach of inclusome is the complex network of drugs included in cyclodextrins, where a link connects two nodes—drugs if they are included in the same cyclodextrin with the same interaction strength level. The emerging inclusome is not scale-free but small-world; we noticed three subcommunities of drugs included by three of the most used cyclodextrins, reflecting drugs classification in the three categories of stability constants (*i.e.*, too weak, optimal, and too strong). Finally, the network based on the structural similarity of guest molecules, built on six PaDel molecular descriptors selected with a genetic algorithm, contains five topological communities, four well-defined and one sparse. The nodes clustered in communities comply with the guest drugs' structural similarity relationship, reflected by the six descriptors, which leads to identifying the relationship between the guests' chemical structure and the formation of the inclusion complex with a particular cyclodextrin. The drug similarity network analysis allows the extraction of potentially relevant structural properties in the inclusion complex formation with a given cyclodextrin. Network science enables the analysis of the drug-cyclodextrin inclusion complex forming process. Indeed, network science has found applications in the medical field, and in recent years a new direction of research has emerged, called network pharmacology, which studies drug pharmacological properties with network science. We used the complex networks specific tools to predict new pharmacodynamic properties for approved drugs. This new methodology, called drug repositioning, considerably shortens the time and costs of discovering new drugs because the preliminary tests are already known and passed. Therefore, we built two types of networks using a technique specific to social networks, which combines the Force Atlas 2 algorithm (which topologically places nodes in the network based on the nodes' gravitational forces interactions) with modularity (which assigns nodes' color). The two complex networks are (*i*) of community-based drug interactions (CBDDIN) and (*ii*) of drug similarity based on the effect on a biological target (DDSN). In CBDDIN, we identified nine topological communities, to which we attributed relevant pharmacological properties to most of the drug nodes. We performed the validation by consulting the literature and other databases and confirmed the predicted properties for 85% of drugs; we consider the remaining 15% repositioning hints because they do not seem to follow the community's label. At the same time, the CBDDIN network centralities classify drugs according to their interaction potential when they are components of various drug treatment schemes. We identified in DDSN 26 topological communities to which we associated specific, dominant properties of drugs; we correctly predicted the pharmacological properties for 86.51% of the drugs, and we consider repositioning hints the remaining 13.49% that do not seem to match the dominant pharmacological property of the community they belong to. The repositioning hints generated by any *in silico* methodology must be further prioritized and tested *in silico* (e.g., by molecular modeling), *in vitro* and *in vivo* for the new pharmacological/pharmacodynamic effect. Hence, we prioritized the candidate molecules

based on the betweenness/degree ratio. We selected azelaic acid (as a potential anticancer drug) and meprobamate (as an antifungal potential) to simulate their interaction with selected biological targets with molecular docking. The molecular docking results showed that the two drugs' interactions with the selected targets are similar to those of some reference drugs, with a known effect on the same targets. We also validated the docking methodology by simulating the interactions between drugs about which there are no data on interactions with the selected biological targets. The results encourage the verification of the new pharmacodynamic effects by *in vitro* and *in vivo* tests.

The last subchapter presents the main scientific contributions achieved by employing network science in respiratory medicine. We analyzed an extensive obstructive sleep apnea syndrome (OSA) patient database using specific complex network tools. Consequently, our main contributions in this regard are the phenotyping of OSA patients and predicting OSA risk with a high specificity score (*i.e.*, SAS_{Score}) based on OSA risk factors. The score, helpful in large patient populations OSA screening, can also be calculated using the application developed for Android mobile phones (<https://play.google.com/store/apps/details?id=aerscore.topindustries.aerscore&hl=en>). As the neck circumference is an OSA indicator, we applied a complex network-based methodology to identify the response patterns to CPAP treatment (continuous positive airway pressure) by associating apnea risk groups with each pattern of response to CPAP treatment. As such, we obtained the neck thickness' threshold value of 41 cm, for which the CPAP treatment is effective. In other words, all patients with a neck thickness higher than 41 cm pertain to the CPAP treatment's best response class. Phenotyping OSA patients by gender using complex networking tools highlights eight specific patient communities/phenotypes for women and men, correlates phenotypes with comorbidities, highlights gender differences, and defines OSA development characteristics.

The following two chapters present the most important academic and professional achievements since graduating from the Faculty of Medicine, the specialization Pharmacy of the "Victor Babeș" University of Medicine and Pharmacy in Timișoara until now, as well as elements of professional activity recognition, reflected by citations in high-impact scientific journals, reviewer activity for scientific journals from prestigious editorial groups and the position of guest academic editor at Pharmaceutics (Q1, IF 4.421).

The last chapter unfolds the academic and scientific development project we undertake after obtaining the habilitation qualification. This project's first plan is the teaching development proposal by diversifying the undergraduate topics, the recommendation of optional and postgraduate courses, and a master's degree program, all inspired by the research topics stated in this habilitation thesis. A second plan is that of the research development proposal, which we intend to achieve by (i) competing in national and international research projects calls, (ii) finding pharmacy students and graduates, as well as other collaborators, for undertaking further research on the

cyclodextrin complexation with both computational and experimental tools, drug repositioning and other related topics from the fields of network pharmacy and network pharmacology, and analysis of respiratory disorders using complex networks. We will achieve these objectives by continuing the interdisciplinary collaboration with the ACSA group members within the Department of Computers and Information Technology of the Polytechnic University of Timișoara (www.acsa.upt.ro); over the years, our collaboration was strengthened by three research project partnerships (one completed, two ongoing), with Professor Paul Bogdan from the University of Southern California, Los Angeles, with Dr. Ștefan Mihăicuță from the Pulmonology Department of the Faculty of Medicine, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, with Professor Radu Mărculescu from the University of Texas at Austin and, of course, with my mentor and doctoral supervisor from the Timișoara Institute of Chemistry of the Romanian Academy – Professor Ludovic Kurunczi. Moreover, we will develop the research infrastructure through ongoing research projects. We will also look for partnerships both in our University and other universities to perform the experiments entailed by the research topics proposed in this habilitation thesis.