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# **PhD THESIS**

**STUDIES IN THE FIELD OF SULFURED HYDROGEN  
AND OF SOME PHYTOCOMPOSITES WITH ACTION  
ON THE CARDIOVASCULAR SYSTEM**

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**ABSTRACT**

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## KEY WORDS:

chili pepper  
ginger  
birch  
mexametry  
polyurethane  
drug delivery ssytem  
tewametry  
garlic  
mistletoe

# **PhD THESIS**

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### **Abstract**

Natural and synthetic chemicals surround us permanently, having a major impact on the environment, but also on our health. There are chemicals that treat or ameliorate various conditions when used rationally, but there are a lot of chemicals that have been classified as toxic even when they reach humans in low concentrations. Toxic substances are already known by the most people. Nowadays, it is necessary to develop modern protection filters, individual protective equipment, safety sensors and to continue the studies in the field of discovering new solutions regarding the antidote in case of intoxications, but especially in the field of prolonged occupational exposure to reduced concentrations of known toxic agents.

In the study of work-related disorders, many countries do not place particular emphasis on the chronic exposure of employees to toxic substances in low concentrations, exposure that does not lead to intoxication or other obvious symptoms immediately. Unfortunately, there are a lot of people who are tired, have a high level of stress, or have cardiovascular symptoms whose source remains unidentified.

Băile Herculane is a resort in the southwest of Romania, recognized worldwide for its springs with sulphurous waters and temperate-mediterranean climate. A very important aspect in the field of *Occupational Health and Safety* is the permanent exposure to gaseous sulfur compounds of the employees in the medical field, who serve the treatment bases. The toxic effects of acute exposure to hydrogen sulfide have been known for decades. However, the number of research investigating adverse health effects due to the low chronic exposure to this toxic agent is limited. Sulfhydryl acid ( $H_2S$ ), known by the scientific world as hydrogen sulfide, is a nasty odorous gas that causes eye and respiratory irritation at low concentrations and becomes fatal at concentrations of about 500 ppm; although  $H_2S$  is naturally occurring in some geothermal and volcanic areas, most of the human exposure comes from industrial processes, such as wastewater treatment plants, paper mills, oil and gas refineries, and from farms for growth. animals. It should not be overlooked that  $H_2S$  is produced at low levels in the human body and has physiological signaling functions. The specialty literature frequently speaks of acute poisoning, accidents at work and even cases of hydrogen sulfide suicide, but

chronic exposures are rarely reported. Thus, research on the cognitive effects of prolonged exposure to low levels of H<sub>2</sub>S is of particular interest.

Regarding the treatment or improvement of some diseases with the help of herbal products, at the current moment of the economic, scientific and industrial development of the developed countries, there is an obvious division of the society between trusts or users on the one hand and skeptics on the other hand. In a world where synthetic pharmaceuticals involve huge costs and long periods from the drug design to the marketing stage, it is natural that their promotion is often done aggressively to boost buyers' confidence. We must not forget, however, that humanity has withstood many natural challenges (pests), even without the availability of synthetic drugs. It is known that the plants have been used since ancient times for their medicinal properties, even from the time of the Egyptians (3,500 years ago), when the onions were used to treat inflamed wounds. Not only in ancient Egypt herbs were used for medical purposes, but also in other early civilizations such as Greece, the Roman Empire, India and China, which enjoyed frequent and widespread use of plants for their medicinal properties.

The development of systems for the delivery / transport of biologically active substances has become one of the most interesting fields of research, located at the border between chemists, biologists, doctors and pharmacists in the last two decades. Particles with sizes between 1 and 5,000 nm were created depending on the targeted receptor, with delayed release, of the micelle or liposomes type, nanofibers or nanotubes, inorganic nanoparticles of hydroxyapatite type, coated with gold or silver, nanoemulsions and nanogels, the studies continuing each year with the improvement of the results already obtained by joint research teams. The multitude of published research papers and the upward trend of their number each year, shows that drug delivery systems are effective in maximizing the therapeutic effect, while reducing the toxicity of biologically active substances.

Regarding the natural compounds with biological activity, besides the multitude of beneficial effects that they possess, the consumption of phytocomposites has never shown adverse effects at the level of those registered in the case of synthetic drugs. Unfortunately, herbal extract medication has not developed enough because there are many classes of phyto-compounds with low stability and solubility, but these impediments can be corrected by making biocompatible formulations that retain their biologically active properties. In order to stabilize the crude extracts, the studies presented in this thesis describe their encapsulation in polyurethane nano- and micro-structures.

In the last four decades, the use of polyurethane has covered many medical fields, such as: cardiovascular devices (catheters, vascular prostheses, cardiac pacemakers), materials for reconstructive surgery

(bandages for wounds, breast implants, maxillofacial prostheses, other implants), obstetrics and gynecology (condoms, contraceptive sponges).

The first part of the thesis, which covers 32 pages, presents in Chapter I. *Descriptive analysis of the knowledge in the domain*, a literature study in which generalities related to hydrogen sulfide are presented - reviewing its discovery, the sources of  $H_2S$ , some physico-chemical characteristics, its impact on the human body and some toxicological data - respectively general informations about the most important phyto-compounds with action on the cardiovascular system. Emphasis is placed both on the history of medicinal plants, on the discoveries of ancient writings from India, China or ancient Greece, as well as on the increasing development of dietary supplements, which are based on human dependence on raw materials of plant nature with which we maintain our health and we heal or improve a number of conditions.

The second chapter contains studies on a group of subjects, employees of the medical system that serves the treatment bases in Băile Herculane resort, a town located in the southwest of Romania, in Caraș-Severin county, Valea Cernei area, known throughout history for its natural factors (an intramontaneous climate, with strong sub-mediterranean influences, iso- and hyper-thermal mineral waters - between 38 and 60 °C - hypotones, with different chemical compositions: sulfurous, chlorinated, calcium, sodium), which favors various treatments, such as aero-therapy, thermal baths in the open basin, respectively sulfurous and salt baths in covered basins, indoor mineral water baths, hydrothermal (and sauna) facilities, electrotherapy, kinetotherapy, in-basin hydrotherapy and inhaler therapy.

Also, the correlations between external factors such as the meteorological parameters (temperature and humidity of the environment, atmospheric pressure and precipitation quantity) and the level of atmospheric hydrogen sulfide on the one hand, respectively the concentration of total urinary excreted sulfur, but also the influence of vitamin B12, a well-known antidote used in  $H_2S$  poisoning represented a real interest.

Quantitative relationships between diet, lifestyle and blood pressure of these human subjects, a computer application for calculating the risk of myocardial infarction and an invention that could prevent the long-term exposure of employees to different concentrations of hydrogen sulphide. are presented successively.

The main contributions of PhD student consist of multiple correlations between the lifestyle of the individuals evaluated on the one hand and the blood pressure values before and after daily exposure to hydrogen sulfide, respectively between the meteorological conditions (temperature, atmospheric pressure, humidity), the level of atmospheric  $H_2S$  and that of the urinary excreted sulfur on the other hand.

The researchers team around PhD student intend to continue the studies described in Chapter II. *Contributions in the field of prolonged exposure to hydrogen sulfide* of this thesis in the following directions:

- the extension of the studies in Băile Herculane area by using a pulse oximeter;
- the evaluation of endothelial cell proliferation in individuals exposed to gaseous hydrogen sulfide compared to a control group;
- the development of a bio-sensor for the detection of gaseous hydrogen sulphide;
- the obtaining of filter compositions for other gaseous sulfur compounds;
- the development of new online and offline applications for determining workplace toxicity and risk of stroke.

In the last chapter, there are presented studies on the obtaining of drug carriers based on organic polymers, which modify the hydrophilic-hydrophobic character of the encapsulated substances and thus improve the transmembrane transfer.

Also, a series of characterization techniques used for nano- and micro-structures used as drug carriers are reviewed: assessments of the products' solubility in various solvents, determination of the samples' pH, technique for analyzing the size and stability of colloidal particles (zetasizer), thermal analysis (differential scanning calorimetry, DSC - use to describe the thermal behavior of samples), spectral techniques (MALDI-TOF, FTIR, UV-Viz), evaluation of the appearance of samples by electron microscopy (SEM) and by modern techniques based on neutron bombardment (SANS), *in vivo* and *in vitro* detection of toxicity of new samples (cytotoxicity and skin irritability studies).

There are presented polyurethane structures used for the transport of natural extracts of chili peppers, ginger, garlic and mistletoe, birch bark, but also the intention to obtain in the future transmembrane transporters used for medicinal substances already established in the treatment of cardiovascular diseases.



Together with the colleagues from our university (Faculty of Pharmacy, respectively the Discipline of Medical Genetics) and those from the Wigner Research Center for Physics Budapest, with whom I collaborated, I consider the extension of these studies for the development of transmembrane carriers used for the encapsulation of pure active substances (APIs) for the treatment of cardiovascular diseases.