STAGE 1 SUMMARY

Our country has huge reserves of biomass, among which the spontaneous flora stands prominently and is one of the most various and least affected by pollution in Europe. Within this spontaneous flora the abundance of medicinal plants is remarkable and is an inexhaustible source of active ingredients which are very useful for the treatment and preventing of some diseases, for personal hygiene items, cosmetic, dyes, etc.

The increasing trend of using herbs in medicine applications is clearly evidenced by research and results already obtained in developed countries, but also in emerging countries such as China, India and Brazil.

The major obstacle in exploiting highly valuable compounds found in medicinal plants from spontaneous flora is that they usually are found in very small quantities and are accompanied by a lot of other compounds, making their extraction and purification very difficult.

Therefore, the amount of research in the field of developing new materials and technological processes deemed as key elements in the industrial extraction of the precious active ingredients found in medicinal herbs has reached a high level worldwide.

An example of such a valuable extract is hypericin which is found in St. John’s Wort, the name being derived from the Latin name of the herb: *Hypericum perforatum.*

Hypericin is considered to have a bright future in the photochemical terapy of skin cancer, but recent studies have proved also other pharmacological effects such as antiviral and antidepressant action.

The same difficulties in the extraction of other active ingredients also exist for this compound. Therefore, it is necessary that innovative technological solutions to be discovered.

The molecular imprinting of polymers is a modern field with an explosive growth and has numerous advantages over separation and purification techniques, such as: easy to prepare, high chemical stability of the biomolecules, tolerance for a wide range of solvents, acids, bases and salts, thermal and mechanical stability, production low cost and the possibility of tailoring the molecularly imprented polymers matrix. Various separation and purification procedures based on this technique are able to offer a fast, simple and selective alternative for the extraction of certain active ingredients from natural products.

The purpose of this project is to develop a technology of obtaining molecularly imprinted polymers with hypericin suitable for its separation from primary extracts. These materials will stand for hypericin efficient selective separation from St. John’s Wort extracts,

in order to manufacture highly bioactive hypericin concentrates. In the same time, both the developed technology and the obtained polymers will have to meet the requirements of industrial application.

Work performed in the first phase of the project included two literature surveys: one related to obtaining molecularly imprinted polymers, physical and chemical characteristics of hypericin, focusing on its tautomer forms, so that the hypotheses in imprinting hypericin would be identified and another one dedicated to the plant named St. John's Wort, its pharmaceutical effects, focusing on the effects of hypericin and on the processes and solvents used to obtain primary hypericin extracts.

The literature surveys have shown that although the enantiomers of hypericin have been separated and studied in order to reflect their chiral properties, the low enantiomerization barrier seems to prevent the occurrence in excess of one of the enantiomers under typical physiological conditions – as long as other chiral entities are absent. Studies carried on, for example, through fluorescence spectroscopy on hypericin complex with chiral biological macromolecules, proteins or enzymes like Human Serum Albumin (HSA) or Glutathione S-transferase have shown that, within experimental errors, the two enantiomers of hypericin produce excitation and emission spectra in stationary regime identical when found in the same chiral environment. Moreover, between the spectra of the two enantiomers there are no differences in any of the systems considered, which led to the conclusion that the spectra and the photo physics of hypericin are in general insensitive to those environments in which it does not form associates.

Circular dichroism spectroscopy applied to the hypericin-human serum albumin complex revealed that the hypericinate (1^-) presents a partial resolution characterized by a 69:31 distribution of the two helical conformers. Further studies to elucidate tautomerism, the conformation and configuration of the enantiomers of hypericin through different techniques like: Magnetic Resonance Spectroscopy (MRS), X-Ray Crystallography, Optical Spectroscopy, Mass Spectroscopy, force fields calculations, etc., have revealed in different stages that the tautomer of hypericin 7, 14 – dioxo (1) is the most stable, with at least 45 kJ / mol, and is found in the composition of hypericin, in acid form or in the form of the corresponding hypericinate ion exclusively or overwhelmingly in both solid state and in polar or nonpolar solvents.

Experimental research consisted in obtaining copolymers of acrylonitrile and in exploring the possibility of turning them into polymer beads. Broadly, there have been identified the main parameters of obtaining the pearls. Meanwhile, research for obtaining molecularly imprinted polymers with hypericin involving the phase inversion technique was initiated.

The copolymers were characterized by FTIR, XPS, TG/DTA and DSC.

Also, experimental research to develop primary hypericin extracts was conducted, varying both extraction solvents and process parameters. The extracts were characterized using several methods.