FINAL REPORT

The research in this project concerned establishing the technology of obtaining molecularly imprinted polymers for the production of hypericine concentrates from St. John's wort.

Two technological procedures for the obtaining of polymeric materials for hypericine separation from an alcohol extract of St. John's wort, especially from pseudo-hypericine, were elaborated: obtaining of polymeric pearls by phase-inversion and obtaining polymeric granules by suspension polymerization.

The original method of phase-inversion yielding hypericine imprinted pearls, presents wide perspectives connected to the economic aspects (regarding a low bioavailability of naphtodianthrones, the lower costs of fito-extracts compared to those of pure compounds) and efficiency-wisely compared to classical separation methods. It was proven that the template (hypericine) was obtained by in situ separation from a fito-extract, previously concentrated and purrified (and containing only hypericine and pseudohypericine).

Also, the original procedure for polymeric granules synthesis, allows for the obtaining of 20-100 μm granules, that can be used in selective adsorption of pseuhohypericine from the primary extract.

Studies done within the project have shown that both technologies could employ natural extracts. Complex analyses indicated the fact that both preparation methods are efficient for a selective separation of naphtodianthrones.

For the products obtained by phase-inversion, hydrodynamic measurements for pore volume estimation were realized, for non-imprinted pearls, as well as imprinted ones, with 5%, 10% extract. Most of the imprinted pearls had a pore volume between 72-90% of the total volume. All imprinted pearls based on copolymers with low content in methacrylic acid (10, 15%, MIP 1 and MIP 2), had larger pore volumes than the corresponding non-imprinted systems. The specific adsorption parameters showed increased adsorption capacities, as well as better imprinting factors in the case of systems containing 25% methacrylic acid (F MIP 4-5=2.89). These results are supported by the selectivity coefficients, reaching maximum values for MIP 2-5 (k~4) and MIP 4-10 (k~3). Moreover, the template concentration played an important role on the pearls' selectivity, the optimum recipe being chosen out of the series bearing a lower fito-extract content (MIP x-5).

In the case of polymeric granules obtained by suspension polymerization, re-adsorption tests indicated the highest adsorption capacities values for the MIP system Itaconic Acid-Acrylic Acid (IA-AA). The Q value in this case for PH is almost double the one for H, being the largest one in both the imprinted and non-imprinted series. These results confirm the reproducibility and accuracy of the adsorption studies on MA/AN based polymers. The maximum F values (>3) correspond also to the MIP IA-AA system, making it the most performant polymer in the studied series.

In order to obtain the concentrated hypericine stocks by the partner PLANTAVOREL, 5 extraction tests were realized at a laboratory scale, using vegetal material with varying hypericine content (0,0431 – 0,0947% g/g p.v), under different extraction conditions, out of which the extraction technology selected was the one corresponding to the test no. 3. This test consisted in a multiple extraction (n=2) of the vegetal product- St. John's wort (*Hyperici flos*).

The validation was realized by using 3 consecutive series of finite products and compared the hypericine concentrate against the specifications in the Technical Data Sheet. The resulted solution, hypericine concentrate from *Hypericum perforatum,* is a complex of naphtodianthrones with a high content in hypericine (min. concentration 0.3% g/g dried substance-obtained values of 0.5-0.57% g/g dried substance); This value of the hypericine concentration (0.5731% g/g dried substance) was used as a base for the information needed to establish the laboratory technology.

By using the proposed extraction technology a hypericine concentrate was obtained, out of St. John's wort (*Hypericum perforatum*). A stability study was realized for the bioactive compound-hypericine, comparing the stored solution against the spray-dried hypericine, kept on a maltodextrine support; the results revealed that the dried concentrated is more stable within the 6 months of the study (2.5% degradation vs. 5.0% in solution state).

A total of 6 ISI articles, with a cummulated impact factor of 13.769 and a BDI indexed article were published during this project. The indicator agreed on was of 4 ISI articles. The articles are uploaded on the platform UEFISCDI-direct. A book chapter was accepted at a renowned publishing house in the USA. From the beginning of the project, 41 scientific communications were presented. The indicator agreed on was 6, therefore being topped almost 7 times. In previous stages of the project, 2 patent applications were filled, the indicator being fulfilled.

During the project 3 PhD theses were realized in the field: Nicolescu (Iordache) Tanta-Verona, Dima Stefan- Ovidiu and Florea Ana-Mihaela. 3 laboratory technologies were realized: technology for obtaining molecularly imprinted pearls with fitoextract by phase-inversion, technology for obtaining imprinted granules with fitoextract by suspension polymerization and optimized technology for preparing hypericine concentrates from St. John's wort. 6 new products were realized: 2 types of imprinted polymeric materials, 5 hypericine extracts, and 3 new procedures for hypericine extraction. In 2013 and 2014, the molecularly imprinted pearls and granules represented ICECHIM at the Research exposition.