STAGE 5 SUMMARY

The 5th stage of the **SPOFLORAHYP** project had as a general objective the **realization of the laboratory technology for the obtaining of granules and proof of the functionality and use of the technology to obtain hypericine concentrates of high bioactivity**.

In order to successfully fulfill the present stage's objective, the research activities followed several converging directions, as follows:

- Obtaining of concentrated hypericine from St. John's wort in order to prove that the separation technology leads to reproductible products; realization of the extraction technology at a laboratory scale.
- Obtaining of hypericine-enriched concentrates and extracts characterization
- Obtaining of hypericine molecularly imprinted polymers by the 2 techniques used in the previous stages
  - Obtaining of polymeric granules by a suspension technology and their morpho-structural and thermal characterization
  - Obtaining of polymeric pearls by phase-inversion and their morpho-structural and thermal characterization
- Establishing of the laboratory technology for the obtaining of hypericine imprinted polymeric granules and proof of the functionality and use of the technology for producing molecularly imprinted polymeric pearls for the selective separation of hypericine.

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*).

For the extraction tests, in order to establish the technology for extracting the total naphtodianthrones expressed in hypericine at a laboratory scale, the following aspects were taken into account:

-the quality of the raw materials (min. 0.8% total naphtodianthrones expressed in hypericine/ parts per volume) is very important to obtain a concentrate with min 0.3% hypericine;

-the preliminary processing of the vegetal material by dechlorophilation and degreasing (Soxhlet/reflux) ensures optimal conditions for hypericine extraction, by removing ballast substances;

-the extraction of integral raw material or processed by extracting the ballast substances, requires the purification of the primary extract (filtration of ballast substances on an talc-like adsorbent);

- homogenity of the vegetal material influences the extraction process and the processe's working parameters. 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).

Regarding the MIP obtaining method, the project coordinator researched various techniques for obtaining molecularly imprinted granules with naphtodianthrones' natural extract from Hypericum Perforatum (fitotemplate).

The studies involved re-adsorption tests for the system based on methacrylic acid (MA) /acrylonitrile (AN), to establish the technology. In this way, large values of Q and F parameters were obtained, proven to be reproductible as well (values are comparable with the previous stages' results). The polymeric system with 75% MA was the one yielding the best results. Morever, a notable result was the one obtained in the case of a 100% MA system, that had the highest pseudohypericine (PH) adsorption. The highest value of k' relative coefficient was also attributed to the imprinted polymer with 100% MA, that competitively adsorbs PH over 8 times more with respect to H and the corresponding non-imprinted polymer. The adsorption properties (F, k, k') are very good. Taking into account the high capacity for pseudohypericine adsorption, Q, this derivate can be extracted, the supernatant being purrified and concentrated in hypericine.

Considering the results of the re-adsorption tests, the technological parameters are validated. Therefore, the technology of obtaining imprinted polymeric granules with hypericine by suspension polymerization was established.

In order to optimize the technology, a study was realized to determine the influence of different comonomers: acrylic acid (AA), hydroxyethyl methacrylate (HEMA), itaconic acid (IA), methacrylic acid (MA), over the molecularly imprinted polymers with fitotemplate, and their morpho-structural, thermal properties and the molecularly imprinting specific parameters. FT-IR analyses confirmed both the imprinting and the template extraction. As predicted, the imprinting is best observed in the case of a 10% extract addition to the matrix. TGA/DTG analyses indicate that the polymers exhibit a good thermal stability, beginning to degrade after 250 °C. A comparison between the imprinted polymers (MIP) and the non-imprinted ones (NIP), revealed similar thermal behaviors, larger differences being noticeable in the case of HEMA-containing granules. In this case, the thermal stability is higher before the extraction, because of the hydrogen bonds that are established between the polymer and the template. SEM analysis showed interesting morphologies because of the smoother surfaces and the absence of broken particles, empty inside compared to the morphology of MA/AN based particles. These conclusions could be the results of the improvement of the extraction method. Re-adsorption tests indicated the highest values of Q for the MIP system AI-AA, with PH adsorption values almost double the ones for H adsorption. These results stand behind the reproductibility and accuracy of adsorption studies for MA/AN based polymers. Also, the maximum values for F (>3) correspond to MIP AI-AA, making this the most effective system out of the studied ones.

Research for obtaining of molecularly imprinted pearls by phase-inversion lead to establishing this innovating imprinting method for the obtaining of macroporous adsorbents with advanced properties, selective for hypericine.

During this stage, studies for the optimization of the laboratory technology for molecularly imprinted pearls were done. They consisted in testing the reproductibility of materials obtained by phase-inversion. Several molecularly imprinted polymeric systems based on AN-MA were obtained, with varying mass ratios: 90:10, 85: 15, 80: 20 si 75: 25, capable of selectively retaining hypericine from primary extracts of *Hypericum Perforatum L.*

The effect of the fitotemplate on the final shape of the pearls was studied by means of reological studies of the copolimer solutions CS x-y, imprinted with extract and non imprinted, at room temperature (25 ^{o}C). Their reological behavior was proven reproductible by its abrupt change with the addition of fitotemplate, from a pseudo-plastic one to a cvasi-Newtonian one. The CS x-y solution prezented the most adequate reology for a reference solution due to its almost Newtonian flow regime. FT-IR analyses confirmed both the imprinting and the template extraction. As predicted, the imprinting is best observed in the case of a 10% extract addition to the matrix. TGA/DTG analyses indicated that all studied polymers are relatively stable; by increasing the methacrylic acid content in the copolymer produces an increase in the weight loss, and a reduced residue quantity at 800 ^{o}C, correspondingly. All polymers except MIP 4-10 present lower mass loss after the extraction of the template, compared with the samples before the extraction, and the non-imprinted ones, weight losses larger than the corresponding imprinted systems. Hydrodynamic measurements for the internal pores volume of the non-imprinted pearls, and the ones imprinted with 5, and 10% extract, were realized. Most of the samples had a pore volume between 72-90% with respect to the total volume. All pearls from copolymers with low percentages of methacrylic acid (10% MIP 1, 15% MIP 2) had larger pore volumes than the corresponding non-imprinted ones. The specific adsorption parameters showed better adsorption capacities and imprinting factors for the system with 25% MA (F MIP 4-5=2.89). It was proven that the adsorption capacities increase with the MA content in the polymeric matrix and decrease with the quantity of added fito-extract. The same effect was noticed in the previous stage, upholding the reproducibility of results. Additionally, the selectivity coefficients, k, have registered maximum values for MIP 2-5 (k~4) and MIP 4-10 (k~3). Moreover, the template concentration had an important role on the selectivity of pearls, the optimum recipe being chosen from the series that used less fito-extract, namely MIP x-5.