STAGE 3 SUMMARY

The research performed within the project was basically aimed at obtaining St. John's Wort hypericin concentrates with enhanced bioactivity.

For this reason research is being conducted on two converging directions: on the one hand a study is conducted on improving the conditions of extraction of hypericin from St. John's Wort with conventional techniques to get phytoextracts with a concentration in hypericin as high as possible and on the other hand these phytoextracts represent the raw material for subsequent concentration carried out by solid phase extraction with molecularly imprinted polymers (MIPs) yielding rich in hypericin (H) phytopreparates.

Researches on the obtaining of molecular imprinted polymer materials with hypericin were developed in the 3rd stage of the project considering the results already obtained in the 2nd phase of the project.

The molecular imprinting of polymers was carried out by two methods:

- Phase inversion imprinting;
- Imprinting by suspension polymerization.

In the phase inversion approach, in order to obtain hypericin molecularly imprinted pearls, four polymer matrices were used, CS x, based on acrylonitrile (AN) and methacrylic acid (MA), with different AN: MA, mass ratios successfully synthesized in the 1st and 2nd stage of the project. CSx powder was dissolved in DMSO, under continuous stirring (200 rpm) in light-protected vials. After 24 h a hydroalcoholic extract of St. John's Wort was added. Through a mechanism dripping, the resulting solutions were poured in the inversion bath which contains water as non-solvent.

Imprinted polymer pearls analyzes were carried out both at ICECHIM and UPB.

Both imprinted and non-imprinted polymers were characterized by FTIR to highlight the imprinting effect on polymer structure and on the efficiency of template extraction from pearls. Because the composition of polymers differ only in terms of mass ratio of monomers and of the amount of extract added before phase inversion, FTIR spectra of the polymers do not undergo major changes. The embedding of naphtodiantrones in pearls and their further extraction were confirmed by the appearance and then disappearance of the -C = O quinone bands, characteristic to H and PH (pseudo hypericin), occurring at about 1660 cm^{-1}.

Surface morphology was investigated for pearls and for interconnected channels with macroporous walls using an Environmental Scanning Electron Microscope (ESEM) FEI Quanta 200 (Philips), equipped with a secondary electron detector in gas medium (GSED), suitable for wet samples. The explanation consists of the fact that copolymer inversion from liquid phase in solid-amorphous phase results in a small decrease in the volume of the polymer (contracting) which creates an effect of exudation of the solvent from the inside of the pearl. This effect generates the macroporous structure of the interconnected channel walls and implicitly the contact with the outer surface through many macropores. Therefore, all the pearls obtained showed a significant volume of pores (68-88% of the pearls volume).

By the second obtaining approach, suspension polymerization process, polymer particles were synthesized using polymer P4 recipe from the previous stage that saw both an imprinted factor above 1 and a maximum selectivity coefficient after conditioning with 0.1 M HCl solution.

Structural XPS analyzes carried out on imprinted and non imprinted particles highlighted the efficiency of the method of extraction with NaOH and conditioning with HCl. Molecularly imprinted polymer particles P4,x and the non imprinted ones P6,x were analyzed by FTIR to highlight the imprinting effect and the efficiency of conditioning with NaOH and HCl. In this case, it was, also, observed, the non-covalent nature of the imprinting, without any additional combination bands or vibration bands characteristic to newly emerging bonds. The structure of the polymers differs only in terms of monomers mass ratio so that their spectra are similar. However, several frequency displacements occurred for C = O and C-OH bonds, which look like they might be involved in non-covalent bonds with the template.

Images recorded on a Gemini® Supra 40VP Scanning Electron Microscope equipped with a secondary electron detector (SE2) showed that the polymers obtained in suspension display macroporous surfaces even in the section. Particle diameters for imprinted polymers are in range of 30-80 μm, being suitable for future applications in solid phase extraction (MISPE). Imprinted polymer surfaces, both before and after extraction and conditioning, are nanostructured and macroporous. Thermal analysis on suspension polymers revealed that all imprinted copolymers, tested before "template" extraction exhibit a single-stage thermal degradation corresponding to the backbone with a maximum speed of around 400 ºC. This attests both the high homogeneity of obtained copolymers and an increase of thermal stability of pendant carboxyl groups (MA) involved in physical interactions with "the template". Thermal degradation occurs approximately totally for all imprinted polymers with wastes amount of maximum 2.5%.

Reference polymers (non imprinted) P 6,x showed similar thermal characteristics to those of the imprinted ones with the appearance of shoulders corresponding to CO_2 release from MA and EDMA before 400 ºC, which appear discreetly around 260 ºC. Determinations for selective separation of H from PH showed that all suspension imprinted polymers exhibit values of above 1 for the selectivity coefficient, k ', whereas for PH suggests a preferential adsorption relative to H. As expected, the higher value of the relative factor k ', was also attributed to the imprinted polymer P 4,1, which adsorbs PH competitively over 132 times more compared to H and non imprinted homologous polymer P 6,1.

Structural analyzes of molecularly imprinted polymers were aimed at correlating the morphology with the adsorption properties. For this reason imprinted polymers and the non imprinted ones were analyzed at different stages of processing prior to extraction, after extraction / conditioning and after final washing step.

In case of imprinted pearls, FTIR analysis revealed the effect of the template introduction by a phase inversion process in an already formed polymer structure. Instead, for the particles prepared in suspension it was highlighted the absence of other newly formed bands, which may have confirmed the occurrence of covalent interactions between monomer and template. XPS analysis of polymer particles attest the surface changes made after conditioning. Thermal properties of the particles are superior to those of pearls obtained in the previous stage. In addition, TGA-DTG analysis confirms the structural changes due to the template and to the conditioning steps.

Laboratory experiments on the extraction of hypericin from St. John's Wort by solid-liquid and liquid-liquid extraction processes (Plantavorel) were conducted in the following directions:

- Selection of plant material - St. John's Wort, flowers - with a high content of hypericin.
- Kinetic studies of hypericin extraction from the whole plant material, in order to determine the optimum extraction parameters.
- Selective extraction studies of hypericin from primary extracts obtained from whole plant material - St. John's Wort, flowers.
- Studies on hypericin extraction from plant material degreasing and chlorophyll removal.

Assessment of hypericin extraction from whole material (St. John's Wort, flowers) resulted in obtaining information on the process parameters and approaching the study on extraction kinetics and on determination of optimal parameters.

Under optimum extraction conditions established by studying the kinetics of extraction, there were conducted **studies on hypericin selective extraction from primary extracts** obtained from whole plant material - St. John's Wort, flowers. Two methods were used:

- Selective extraction of hypericin, from primary extract with different polarity solvents (petroleum ether and ethyl acetate);
- Chlorophyll elimination and degreasing from primary extract with adsorbent materials (talc, magnesium stearate, magnesium oxide).

It was found by UB PROTMED partner that the extraction sequence including two steps degreasing with a mixture of Et_2O and EtOAc and a larger amount of water leads to an optimal yield of naphtodiantrones recovery.

Microwave-Assisted Extraction (MAE) is an attractive alternative compared to conventional methods such as Soxhlet extraction, percolation, digestion, extraction under reflux, sonication and steam distillation. The high temperatures reached upon microwave heating of the extraction dramatically reduce both the extraction time and the required amount of solvent.

There were analyzed a series of extracts from St. John's Wort coming from adsorption tests conducted on molecularly imprinted polymers prepared by the two methods. From the achieved studies it seems that suspension preparation method is superior to phase inversion.