Stage I (2020): Preliminary synthesis of Fe_XOy-CTAB powders

Summary of the stage: During this stage, the preliminary synthesis of FexOy-CTAB powders was achieved by using an adapted method. The obtained powders were analyzed by physical-chemical methods such as: X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), DLS (Dynamic Light Scattering) studies. The preliminary results obtained from the DRX studies revealed the purity of the investigated sample, the average size of the estimated FexOy-CTAB crystallite was 26 ± 3 nm. TEM and SEM images highlighted the spherical morphology and the nanometer size of FexOy-CTAB. The functional groups present in the M-CTAB powders have been revealed by FTIR studies. Preliminary DLS results highlighted the presence of a bimodal distribution. During this stage, it was established a protocol concerning the realization of the *in vivo* experiments. Arsenic adsorption from contaminated aqueous solutions using iron oxide nanoparticles coated with cetyltrimethylamonium bromide (FexOy-CTAB) will be achieved by adsorption experiments on batch level.

During this stage, it was developed a dedicated website for the research project (https://febioremove.proiectecercetaredezvoltare.ro/index.php/ro/), with the participation of both partners (UASVMB and NIMP).

In conclusion, the objectives and activities proposed for Stage 1/2020 have been fully achieved.

Step II (2021): FexOy-CTAB powder's obtaining technology. Removal of arsenic ions from contaminated solutions. Assessment of arsenic toxicity to vital organs of rats

Summary of the stage: Based on the results obtained within Act 1.1 (Preliminary synthesis regarding the obtaining of FexOy –CTAB powders), within this stage it was developed a protocol regarding the method of obtaining FexOy powders functionalized with cetyltrimethylammonium bromide (CTAB) (FexOy-CTAB). Both the iron oxide powders functionalized with CTAB and the iron oxide powders functionalized with CTAB recovered after the decontamination experiments were characterized from the physical-chemical point of view by using several methods. The DRX studies performed on the analyzed samples have highlighted their purity as well as the fact that they are made up of a single phase. Thus, in the case of iron oxide powders functionalized with CTAB, it was obtained an average size of about 18 nm and for the iron oxide powders functionalized with CTAB recovered after the decontamination experiments, an average size of about 15 nm. In the TEM micrographs obtained on the analyzed powders it can be noticed that the powders are made up of nanoparticles with spherical morphology. In SEM micrographs it can be seen that the powders are made up of particles with nanometer dimensions and with a spherical morphology. Also, the EDX spectra confirm the presence in powders of the main constituent chemical elements, namely: O, N, Br and Fe (for iron oxide powders functionalized with CTAB) O, N, Br, Fe and As (for iron oxide powders functionalized with CTAB recovered after decontamination

experiments). Thus, the EDX spectra confirm the purity of the powders and in the case of iron oxide powders functionalized with CTAB recovered after the decontamination experiments, it was confirmed the adsorption of As. The presence of vibration bands characteristic to iron oxides butalso to CTAB was highlighted by Fourier transform infrared spectroscopy (FTIR) studies. During this phase of the research project, a laboratory protocol was also developed for the removal of As^{3+} ions from contaminated water using iron oxide powders functionalized with CTAB. Thus, the adsorption capacity of As^{3+} ions using FexOy-CTAB powders was studied by conducting batch experiments and quantifying the concentration of As^{3+} in decontaminated aqueous solutions. The obtained results highlighted the fact that the material used as an adsorbent (iron oxide functionalized with CTAB) has a strong affinity towards the As(III) ions. The results of the evaluation of arsenic toxicity on the vital organs of rats highlighted the toxic character of arsenic on the studied organs. The results of assessing the general health state of rats after oral administration of water contaminated with arsenic showed that the general condition of rats began to show slight changes. It has also been observed that the exposure of rats to arsenic leads to a change in the parameters of both the blood count and the liver and kidney function, respectively. The results of the histopathological microscopic examination in the case of kidneys and liver collected from rats fed with decontaminated water using iron oxide powders functionalized with CTAB, clearly highlight their nontoxic character on the investigated organs. In the visual exam, it could be noticed that after the administration of the water decontaminated with iron oxide functionalized with CTAB, the general condition of the rats did not undergo any visible changes compared to that of the rats in the reference group. Also, there were not observed important changes in the parameters of the blood count as well as of the liver, and kidney function respectively.

In **Step 2/2021** we published **a work** in an ISI-rated journal and **sent for publication an article to an ISI-rated journal.** Also, 1 paper was presented **at a scientific event**. During this stage, working visits were held in different laboratories abroad for the realization and validation of some physical-chemical measurements (according to the project's realization plan). During this stage, the website dedicated to the research project was updated, with the participation of both partners (USAMVB and INCDFM).

In conclusion, the objectives and activities proposed for Stage 2/2021 have been fully achieved.

Step III (2022): Verification of the repeatability of experimental results

Summary of the stage: In this research project, our studies were directed towards the development of iron oxide powders functionalized with CTAB and with an increased efficiency in removing arsenic ions from contaminated waters. According to the results obtained by the determination of Langmuir and Freundlich constants, the processes of adsorption of arsenic ions by iron oxide powders functionalized with CTAB were favorable, and the iron oxide powders functionalized with CTAB could be successfully used in the future for the development of new technologies regarding the remediation of

contaminated waters. During this stage, studies were also carried out to evaluate the repeatability of experiments with regard to *the in vivo* toxicity of arsenic to vital organs of rats. The results of histopathological studies indicate the toxicity of arsenic ions present in contaminated waters on the liver and kidney of rats. Moreover, histopathological images indicate the absence of structural changes in the case of vital organs (kidneys and liver) of rats fed with decontaminated water using iron oxide powders functionalized with CTAB. These results are very similar with those obtained in the case of vital organs of rats fed with water contaminated with arsenic / decontaminated water using iron oxide powders functionalized with CTAB (within the II stage).

In **Stage 3/2022** we published **6 scientific papers** in ISI rated journals and **sent for publication an article to an ISI-rated journal.** Moreover, 1 paper was presented **at a scientific event**. Also, it was made the drafting and the submission to OSIM of an **application for a patent** (No. A 2022 0066). During this stage, working visits took place in different laboratories in France. The update of the website of the research project was also done.

In conclusion, the objectives and activities proposed for Stage 3/2022 have been fully achieved.

The scientific reports will not be presented on the website of the project, the results still being in the ongoing process of publication.