Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVI

Purification and Characterization of Iron Oxide Nanoparticles (IONs) and their potential use as Drug Delivery Vectors

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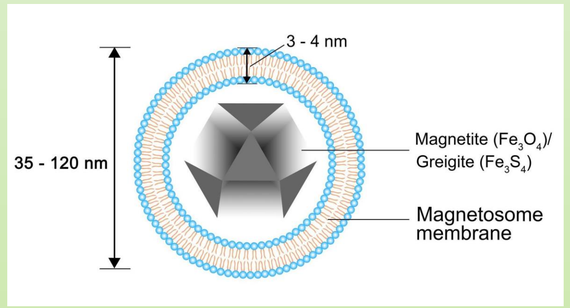
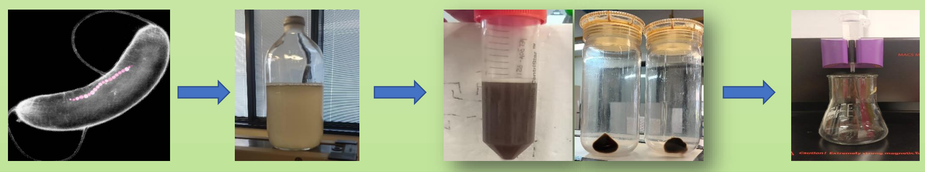
Introduction

Iron Oxide Nanoparticles (IONPs) are widely used as drug delivery agents in the treatment of many diseases and drug delivery processes. The dimensions, the morphology, the characteristics of the surface and the type of material used are important aspects to consider for a tailored functionalization of NPs on the basis of their use. The process of synthesizing pure and uniform NPs is a quite expensive and time-consuming. An innovative approach to overcome these problems is the use of naturally synthesized NPs, which are isolated from bacteria. In the PhD project, we propose to use iron-NPs naturally produced by **Magnetotactic bacteria (MTB)**. These IONPs, also called **magnetosomes**, are formed by a core of iron oxide (Fe3O4) coated with a lipid bilayer filled with membrane proteins. This protein rich bilayer could provide large surface and binding pockets for the attachment of many desired drugs or bioactive molecules. Currently we are characterizing these IONPs purified from Magnetospirillum gryphiswaldense by means of Small Angle Neutron Scattering (SANS),Small Angle X-Ray Scattering (SAXS), Dynamic Light Scattering (DLS), Atomic Force Microscopy (AFM), X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM).



1. Purification of IONPs from MTB

1. Culturing of Magnetospirillum gryphiswaldense in microaerobic conditions
2. Lysis of cells through centrifugation and sonication to obtain magnetosomes
3. Purification of IONPs (magnetosomes) by Magnetic separation column

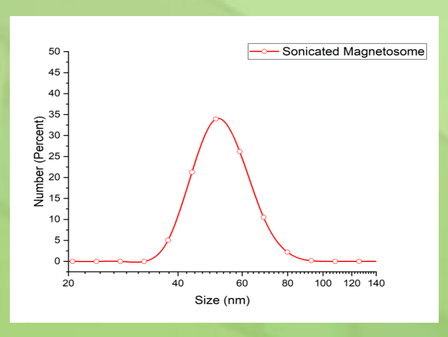


Step 1. Growing MTB — Step 2. Extraction of Magnetosmes — Step 3.Purification by MSC

2. Preliminary Analysis

Dynamic Light Scattering (DLS)

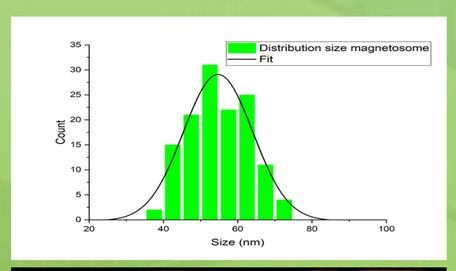
DLS measurements were done using a Malvern Zetasizer PRO operating with a fixed angle of 173°. The temperature was at 25° C. The diameter of the magnetosomes results around 35-55 nm, with a poly dispersion index (PDI) of 20%.

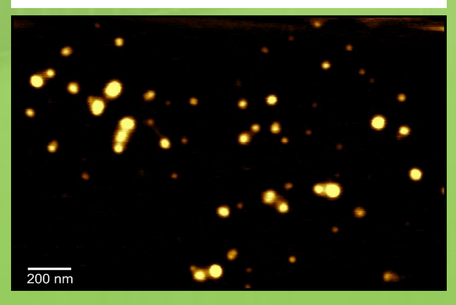


Atomic Force Microscopy (AFM)

AFM measurements were carried out on an AIS-NT's Scanning Probe Microscopy, (Horiba Scientific).

All images were acquired at resolution of 512×512 pixels, with a scan rate of 1 Hz and were analyzed with the Gwyddion and ImageJ (version 1.8.0) software. A statistical measurement of the average particle diameter was performed on a large enough number of particles (>80). The average diameter resulted 54.7 nm with PDI 18.6.



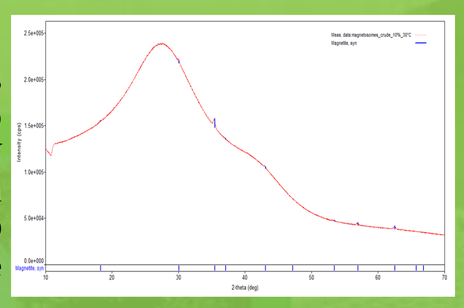


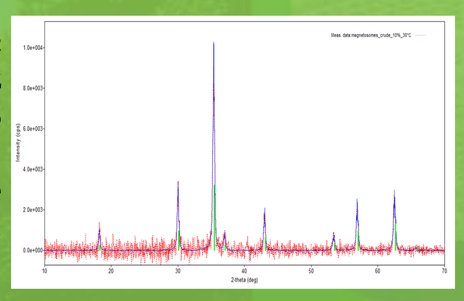
X-Ray Diffraction (XRD)

Crystalline structure of magnetosomes was analyzed by RIGAKU SmartLab Diffractometer featuring a 9 kW rotating anode X-ray generator, a HyPix-3000 high-energy-resolution 2D semiconductor detector, an in-plane diffraction arm.

The crude samples grown in 10% O_2 concentration were loaded in quartz capillary tubes with a diameter of 1.5 mm and exposed to X-rays.

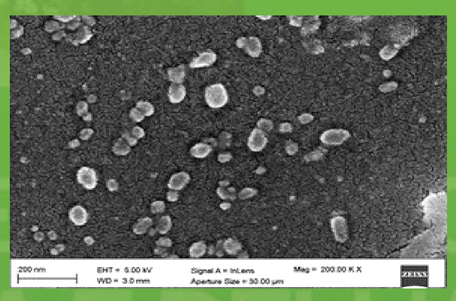
The peaks for magnetite (Fe3O4) were recorded at 19.1°, 31.7°, 43.3°, 55.9°, 57.5° and 63° respectively.





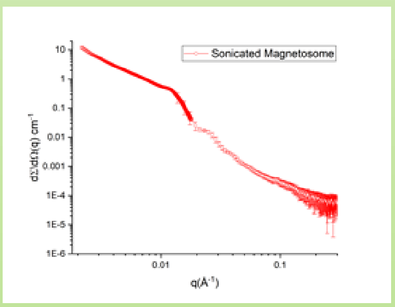
Scanning Electron Microscopy (SEM)

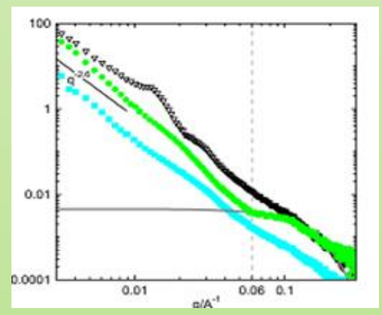
SEM analysis was done on SEM PHILIPS XL20 with filament W and a maximum voltage of 30 V. Results were confirmed by SEM which also showed the particle size ranges from 40-100 nm



3. Small Angle X-Ray Scattering (SAXS)

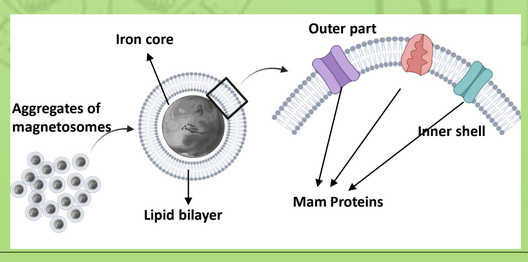
SAXS curve recorded at ESRF, The European Synchrotron (Grenoble, France) in Beam-line ID2. Magnetosomes prepared in 70 mM PBS solution and kept at 4° C. The curves were recorded at 25°C. Comparison of the SAXS curve of our sample (Red) with the ones published by Sabine et al. (2021) (blue and black)

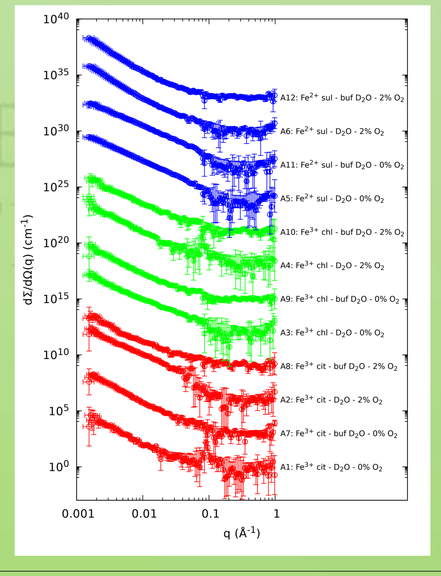




4. Small Angle Neutron Scattering (SANS)

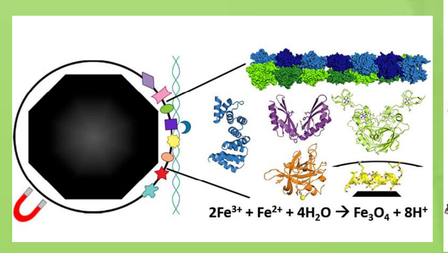
The SANS experiments were performed in the SANS2d instrument at the ISIS neutron source (Didcot, UK). Samples of magnetosomes prepared under different conditions and dissolved in heavy water were measured. SANS data are shown in the Figure. Each curve has been scaled for clarity. SANS data analysis is in progress. The model we are working on involves the formation of clusters of magnetosomes, as shown in the Figure.

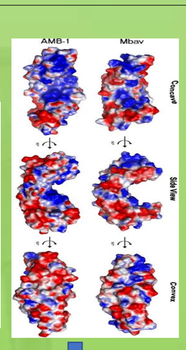


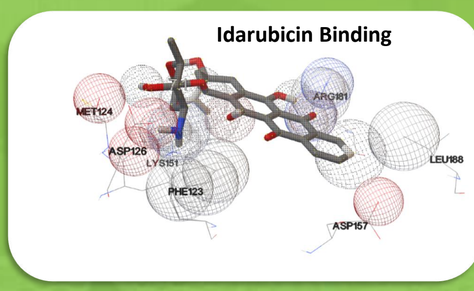


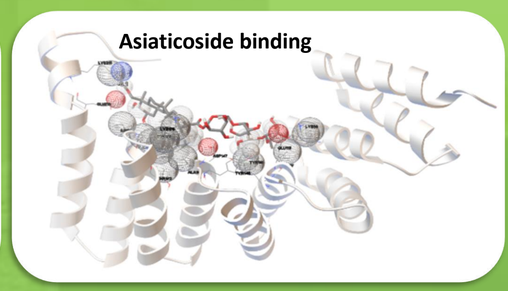
5. Application of Magnetosomes In Drug Delivery

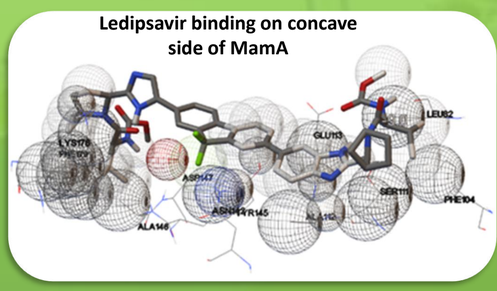
- More than 80 proteins have been identified in magnetosomes (MS) which are integrated in the lipid bilayer around the iron core
- Most abundant protein is MamA, 24KD
- MamA has concave and convex side with binding sites for bioactive molecules
- Software used for VS were Autodock Vina and Chimera for protein stabilization











6. Analysis to be Performed for Drug docking confirmation

Nano- IR Spectroscopy

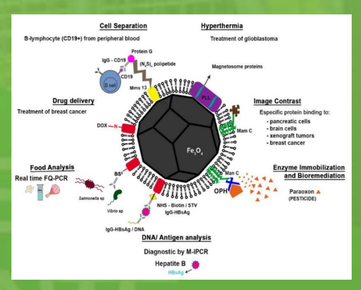
The Nano-IR spectroscopy investigation will shed new light on the chemical composition of the external surface of magnetosomes that includes the lipid bilayer and proteins.

The aim is to experimentally confirm the interaction obtained by Molecular Dynamics simulation of methotrexate, doxorubicin and ifosfamide.

Experimental sample	Composition
1	Magnetosome
2	Magnetosome
3	Magnetosome
4	Magnetosome + Methotrexate
5	Magnetosome + Doxorubicin
6	Magnetosome + Ifosfamide

Future application of Magnetosomes

- Hyperthermia
- Cell separation
- MRI
- DNA analysis
- Bioremediation



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