ISIS@MACH ITALIA

Science and Technology Facilities Council
ISIS Neutron and Muon Source

Experiment Proposal

Experiment number GP2024024

Principal investigator (*)	Dr Rita Gelli, University of Florence & CSGI, ITALY
Co-investigator	Professor Francesca Ridi, University of Florence & CSGI, ITALY
Co-investigator	Miss Debora Briganti, University of Florence, ITALY
Co-investigator	
Co-investigator	
Co-investigator	
Co-investigator	
Co-investigator	
Co-investigator	
Experiment title	Characterisation of polyacrylic acid-stabilised amorphous magnesium-calcium phosphate nanoparticles through transmission electron microscopy
MRF Instrument	**TEM JEOL**
Access Route	Direct Access
Science Areas	Chemistry, Materials
Sponsored Grant	None
Grant Title	-
Start Date	-
Similar Submission?	-
Industrial Links	-

Days requested: 3
Previous GP Number: No
DOI: -
Sponsor: -
Grant Number: -
Finish Date: -

Non-Technical Abstract Amorphous calcium-magnesium phosphate nanoparticles (AMCPs) represent interesting platforms for drug delivery and nutraceutics due to their high availability, low costs, bioavailability, biodegradability, drug loading capacity and responsiveness to stimuli. Their ability in drug loading, delivery and their cellular uptake is intimately connected to the size, and the inclusion of specific molecules and polymers during the synthesis can modulate their dimensions and thus their efficacy. This project focuses on the preparation of AMCPs with tunable size and variable Ca-Mg content stabilized by polyacrylic acid. The presence of different amounts of Mg in the particles is fundamental to stabilize the amorphous phase and prevent crystallization. By optimizing different synthetic parameters we succeeded in modulating particles' size from some microns to tens of nm. Transmission electron microscopy would be fundamental to characterise the morphology and porosity of the obtained nanoparticles.

Publications -

ISIS neutron and muon source

E-platform: No

Instruments	**Days Requested:**
Access Route	**Previous RB Number:**
Science Areas	**DOI:**
Sponsored Grant	**Sponsor:**
Grant Title	**Grant Number:**
Start Date	**Finish Date:**
Similar Submission?	
Industrial Links	

Sample record sheet

Principal contact Dr Rita Gelli, University of Florence & CSGI, ITALY
MRF Instrument **TEM JEOL** **Days Requested:** 3
Special requirements:

SAMPLE

Material	Calcium-Magnesium phosphate nanoparticles stabilized by polyacrylic acid	Calcium-Magnesium phosphate nanoparticles stabilized by polyacrylic acid	Calcium-Magnesium phosphate nanoparticles stabilized by polyacrylic acid
Formula	CaMgHx(PO4)y + (C3H4O2)n	Ca3Mg2Hx(PO4)y + (C3H4O2)n	Ca2MgHx(PO4)y + (C3H4O2)n
Forms	Friable powder	Friable powder	Friable powder
Volume	cc	cc	cc
Weight	5 mg	5 mg	5 mg
Container or substrate	Powders will be dispesed in ethanol, sonicated and a drop will be deposited on a TEM grid	Powders will be dispesed in ethanol, sonicated and a drop will be deposited on a TEM grid	Powders will be dispesed in ethanol, sonicated and a drop will be deposited on a TEM grid
Storage Requirements	-	-	-

SAMPLE ENVIROMENT

Temperature Range	270 - 280 K	270 - 280 K	270 - 280 K
Pressure Range	- - - mbar	- - - mbar	- - - mbar
Magnetic field range	- - - T	- - - T	- - - T
Standard equipment	None	None	None
Special equipment	No special equipment is requested	No special equipment is requested	No special equipment is requested

SAFETY

Prep lab needed	Yes	Yes	Yes
Sample Prep Hazards	No other hazards associated with the sample preparation	No additional hazards associated with the sample preparation	No additional hazards associated with the sample preparation
Special equip. reqs	An ultrasonic bath to place the vials to properly disperd the nanoparticles	An ultrasonic bath to place the vials to properly disperd the nanoparticles	An ultrasonic bath to place the vials to properly disperd the nanoparticles
Sensitivity to air	No	No	No
Sensitivity to vapour	No	No	No
Experiment Hazards	No other hazards associated with the experiment	No other hazards associated with experiment	No other hazards associated with experiment
Equipment Hazards	-	-	-
Biological hazards	No biological hazards associated with the sample	No biological hazards associated with the sample	No biological hazards associated with the sample
Radioactive Hazards	No radioactive hazards associated with the sample	No radioactive hazards associated with the sample	No radioactive hazards associated with the sample
Additional Hazards	-	-	-
Additional Details	-	-	-
Sample will be	Disposed by IS	Disposed by IS	Disposed by IS

Science case for ISIS@MACH ITALIA Experimental Proposal

"Characterisation of polyacrylic acid-stabilised amorphous magnesium-calcium phosphate nanoparticles through transmission electron microscopy"

1. Background and Context

Nutraceutics is a multidisciplinary field that uses bioactive substances from food and plants to prevent and treat a wide range of disorders and diseases due to their beneficial properties (such as antibacterial, antiviral and antitumor effects).[1] The interest in nutraceutics stems from various advantages including public acceptance, lower costs and side effects compared to traditional drugs and potential synergies with conventional therapies. Scientific research focuses on increasing bioavailability through controlled release systems (drug delivery). Amorphous Magnesium-Calcium Phosphate nanoparticles (AMCPs) are promising candidates as biomimetic carriers for the delivery of nutraceuticals and conventional drugs due to their peculiar properties: presence in the ileum (where they are involved in immune surveillance), bioavailability and biodegradability, drug loading capacity, responsiveness to stimuli (e.g. pH), functionalization capability, high availability and low costs.[2,3] Despite the benefits of using AMCPs, they spontaneously tend to crystallise and, due to their spontaneous micrometric size, experience limited cellular uptake. Hence, it is crucial to identify methodologies to increase Mg content (known to delay amorphous-crystalline conversion) and reduce AMCP size to the nanoscale for enhanced cellular uptake.[4,5] We developed AMCPs with different Ca/Mg ratios stabilised with polyacrylic acid (PAA) of different molecular weights, having a size of 80-120 nm. To characterise the morphology and porosity of AMCPs, it would be fundamental to perform Transmission Electron Microscopy measurements that allow excellent resolutions even with nanoscale particles. This work is part of the PLANTFORM project funded by MIMIT (Ministry of Enterprises and Made in Italy) which involves the University of Florence and two important Italian biotechnology industries. The goal of the project is to develop delivery systems for nutraceutics. Additionally, several researchers, fellowship holders and students from the University of Florence are involved in the project.

2. Proposed experiment

The aim of the experiment is to characterise the morphology of AMCPs stabilised with PAA at different Ca-Mg content. Attempts to define their morphology were made using a Scanning Electron Microscope (SEM); however, due to their limited size, it was not possible to obtain images with adequate resolution (Fig.1). We would like to use the TEM JEOL available among the ISIS@MACH ITALIA instruments for this purpose. Samples can be prepared dispersing the freeze-dried powder in ethanol following sonication.

3. Summary of previous experimental proposals or characterisation

Samples with different Ca/Mg ratios (ranging from 0.2 to 0.8 from A to D) stabilised with PAA of varying molecular weights have been prepared, yielding to stable nanoparticles of 80-120 nm (Fig.1) and remain stable even after lyophilization and re-dispersion in water. The amorphous nature and the presence of PAA have been confirmed through Fourier Transform Infrared Spectroscopy (FTIR).

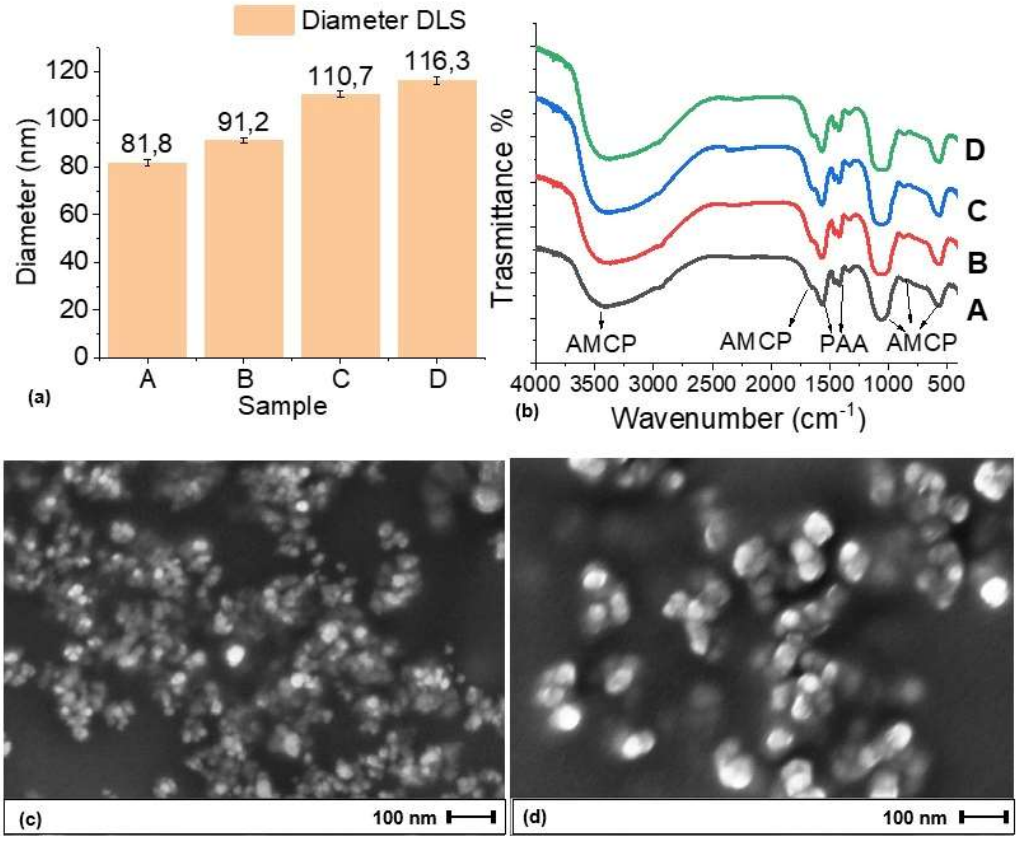

***Fig.1**: (a) Dynamic Light Scattering diameters, (b) FTIR spectra of AMCPs, (c,d) SEM micrographs of sample C and D, respectively.*

4. Justification of experimental time requested

We aim at studying 10 AMCP samples with different Ca/Mg ratio and prepared with PAA of different molecular weight. As suggested by the instrument scientist, taking into account samples deposition on the grid, their loading in the microscope and the instrument setup, three days of measurement would be necessary.

References

1. Gonçalves et al. *Trends Food Sci Tech* 78, 270–291 (**2018**).
2. Powell et al. *Nature Nanotech* 10, 361–369 (**2015**).
3. Khalifehzadeh et al. *Adv Colloid Interface Sci* 279, 102157 (**2020**).
4. Gelli et al. *J Colloid Interf Sci* 531, 681–692 (**2018**).
5. Guo et al. *J Nanobiotechnol* 19, 32 (**2021**).