ISIS@MACH ITALIA

Science and Technology Facilities Council
ISIS Neutron and Muon Source

Experiment Proposal

Experiment number GP2023088

Principal investigator (*)	Mr Pietro Tordi, University of Florence & CSGI, ITALY
Co-investigator	Dr Rita Gelli, University of Florence & CSGI, ITALY
Co-investigator	Professor Francesca Ridi, University of Florence & CSGI, ITALY
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Experiment title	Study of the internal structure of alginate fibers crosslinked with different cations by confocal laser microscopy
Training MRF	**Confocal Microscope 3**
Access Route	Direct Access
Science Areas	Chemistry, Materials
Sponsored Grant	None
Grant Title	-
Start Date	-
Similar Submission?	-
Industrial Links	-

Days requested: 2
Previous GP Number: No
DOI: -
Sponsor: -
Grant Number: -
Finish Date: -

Non-Technical Abstract

Alginate (Alg) is a biocompatible and biodegradable anionic polysaccharide with high application potential which can be easily shaped into hydrogel fibers using an extrusion and crosslinking strategy, taking advantage of different metal cations. Those fibers are of interest in a number of applications including wound healing, water purification and flame retardancy. Preliminary investigations suggest that fibers from different cations have a characteristic internal morphology, due to the specific diffusion and crosslinking. The goal of this training is to learn how to investigate the internal structure of swollen Alg fibers crosslinked with different cations using confocal laser microscopy. Such technique will allow for the observation of the inner structure of Alg fibers in the swollen state at the micro-scale, upon staining the polymer with a fluorescent probe. The obtained results will relate the features of the internal structure of the fibers with their macroscopic properties.

Publications -

ISIS neutron and muon source

E-platform: No

Instruments	
Access Route	
Science Areas	
Sponsored Grant	
Grant Title	
Start Date	
Similar Submission?	
Industrial Links	

Days Requested:
Previous RB Number:
DOI:
Sponsor:
Grant Number:
Finish Date:

Sample record sheet

Principal contact Mr Pietro Tordi, University of Florence & CSGI, ITALY
Training Instrument **Confocal Microscope 3** **Days Requested:** 2
Special requirements:

SAMPLE

Material	-	-	-
Formula	-	-	-
Forms			
Volume			
Weight			
Container or substrate	-	-	-
Storage Requirements	-	-	-

SAMPLE ENVIROMENT

Temperature Range	-	-	-
Pressure Range	-	-	-
Magnetic field range	-	-	-
Standard equipment	-	-	-
Special equipment	-	-	-

SAFETY

Prep lab needed	-	-	-
Sample Prep Hazards	-	-	-
Special equip. reqs	-	-	-
Sensitivity to air	-	-	-
Sensitivity to vapour	-	-	-
Experiment Hazards	-	-	-
Equipment Hazards	-	-	-
Biological hazards	-	-	-
Radioactive Hazards	-	-	-
Additional Hazards	-	-	-
Additional Details	-	-	-
Sample will be	-	-	-

Training Case for ISIS@MACH ITALIA Experimental Proposal
"Study of the internal structure of alginate fibers crosslinked with different cations by confocal laser microscopy"

1. Background and Context

Alginate (Alg) is a biocompatible and biodegradable anionic polysaccharide with high application potential due to its reactivity and selectivity towards metal cations. The hydroxyl and the carboxylate groups on the polymer backbone are involved in the coordination of divalent metal cations, producing crosslinked hydrogels with characteristic porosities and mechanical properties. We developed a facile extrusion and crosslinking approach for the preparation of M^{2+}-crosslinked Alg fibers, which can reach lengths up to some meters thanks to the optimization of the processing parameters. Furthermore, the type of cation and the related complexation geometries allow one to obtain fibers suitable for several applications, including wound healing, water purification and flame retardancy. The diffusion and crosslinking processes responsible for the fiber-formation still need to be unraveled, although their knowledge is crucial for the design of systems with finely tuned properties. This study is part of Pietro Tordi's research activity as a PhD student in co-tutorship between the University of Florence (Italy) and the University of Strasbourg (France), funded by the Italian Ministry of University and Research (MUR) for three years. The aim of the project is the realization of Alg-based composites for wound healing, water/air purification and pressure-based sensors. Part of the studies are currently being carried out at the *Institut de Science et d'Ingeniérie Supramoléculaires* (ISIS, University of Strasbourg), in the Nanochemistry Lab of Prof. Paolo Samorì. The in-depth characterization of the self-assembly properties of Alg in the presence of cations will be beneficial for the preparation of 2D Alg-graphene pressure-sensitive devices.

2. Proposed Training

People to be trained include a PhD student (Pietro Tordi), a researcher (Dr. Rita Gelli), and a professor (Prof. Francesca Ridi), working at the Department of Chemistry of the University of Florence and interested in learning the potentialities of confocal microscopy for the characterization of hydrogels, in particular Alg-based ones. Confocal laser microscopy represents a remarkable tool to unravel the inner structure of Alg fibers at the micrometric scale, as it allows for the observation of the hydrogel in the swollen state and does not require any drying procedure that could lead to artifacts in the structure. MRF staff members contacted in advance prior to the submission will carry out the training.

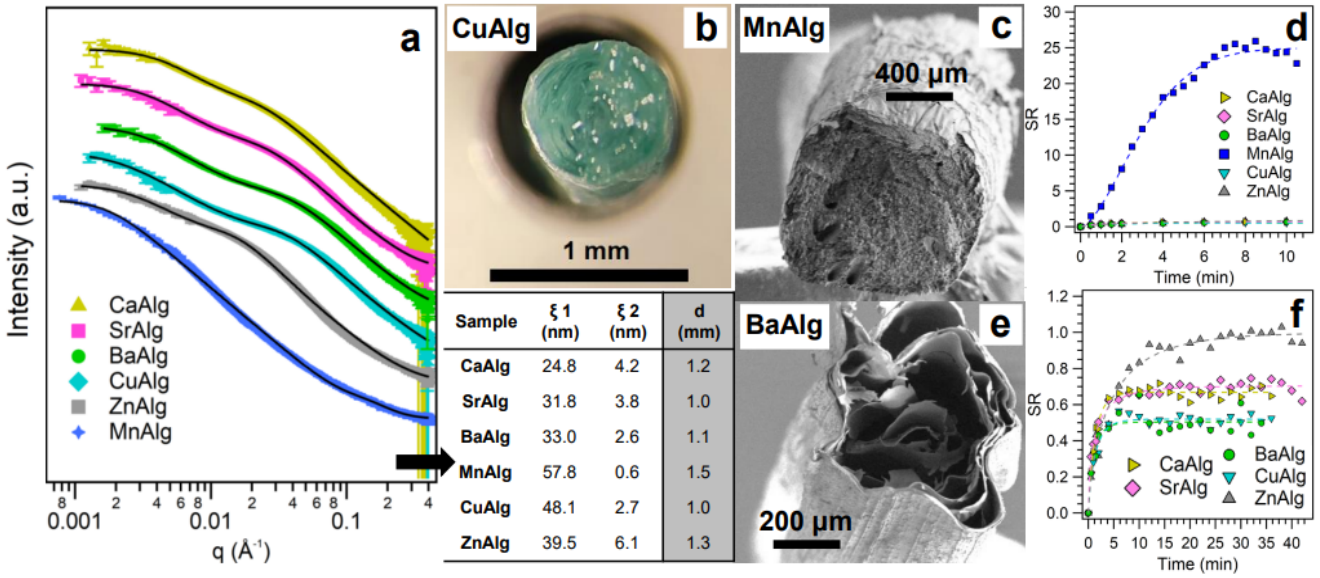
3. Summary of previous training proposals

No training proposals have been previously submitted through the ISIS@MACH ITALIA infrastructure; nevertheless, we already had the possibility of studying Alg fibers crosslinked with different cations through Small Angle X-ray Scattering experiments, thanks to the allocation of beamtime in the Call for Direct Access round 23-1 (proposal GP2023028). Such characterization led to interesting results (see Section 4, Fig. 1a), and is expected to contribute to the publication of a scientific paper.

4. Justification of experimental proposals request

The aim of the experiment is to observe the internal morphology of swollen Alg fibers crosslinked with various cations (Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+}) by means of confocal laser microscopy.

It is believed that different cations impart a different structural organization to Alg chains, due to their different size/charge/coordination geometry. As highlighted by our results, a different assembly at the nanoscale (SAXS, Fig. 1a) results in significant variations in the fibers properties (*e.g.* morphology [c,e] and water absorption [d,f]). The characterization of the internal structure of the swollen fiber at the microscale, possible with confocal laser microscopy, would be fundamental to clarify the diffusion process of the different ions within the hydrogel matrix and the crosslinking process, eventually relating the features of the internal structure of the fibers with their macroscopic properties.



Sample	ξ 1 (nm)	ξ 2 (nm)	d (mm)
CaAlg	24.8	4.2	1.2
SrAlg	31.8	3.8	1.0
BaAlg	33.0	2.6	1.1
MnAlg	57.8	0.6	1.5
CuAlg	48.1	2.7	1.0
ZnAlg	39.5	6.1	1.3

Figure 1. (**a**) SAXS curves of M^{2+}-Alg fibers. (**b**) Swollen Cu^{2+}-Alg fiber's cross-section. SEM micrographs of the lyophilized Mn^{2+}-Alg (**c**) and Ba^{2+}-Alg (**e**) cross-sections. (**d,f**) Swelling profiles of the M^{2+}-Alg samples. * Characteristic correlation lengths (ξ) and swollen diameters (d) of the M^{2+}-Alg samples are reported in the table.

The training would allow us to answer some fundamental questions about the structure of Alg fibers, namely: *i)* Is it possible to observe a different internal structure of Alg fibers depending on the cation used to crosslink the matrix? *ii)* If the fiber is crosslinked with a cation that imparts a specific structure, is it possible to exchange the crosslinking cation and follow in real time the modification of the structure?
Our samples consist of six swollen Alg fibers crosslinked with Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+}, having diameters ranging from 1 to 1.5 mm. Considering that the field-of-view of Leica TCS SP5 II is 750 µm, the possibility of acquiring in tile scan mode will be taken into account. Alg can be fluorescently-labeled with a probe interacting with the carboxylic units on the polymeric backbone, in order to distinguish between the different porous/layered structures of the Alg fiber. Prior to the experiments, staining tests with different fluorescent probes will be performed at the University of Florence, following the advice of the instrument scientist, in order to determine the most suitable probe in terms of stability and binding conditions. As suggested by the instrument scientist, the attention will be devoted to fluorescent probes with excitation wavelength close to 488 nm (according to the literature, fluorescein isothiocyanate (FITC) might be a promising candidate). To carry out the training and the subsequent analysis of the six samples, a total of 2 days of operation is required (safety considerations: 1 h, instrument set-up: 1 h, measurement run: 2 h for each sample, total 12 h, data analysis: 2 h).