ISIS@MACH ITALIA

UKRI Science and Technology Facilities Council ISIS Neutron and Muon Source

Experiment Proposal

Experiment number GP2023029

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Experiment title Study of the internal structure of alginate fibers crosslinked with different cations by means of FIB-SEM

MRF Instrument	**FIB-SEM GAIA 3**	**Days requested:** 1
Access Route	Direct Access	**Previous GP Number:** No
Science Areas	Chemistry, Materials	**DOI:** No
Sponsored Grant	None	**Sponsor:** -
Grant Title	-	**Grant Number:** -
Start Date	-	**Finish Date:** -
Similar Submission?	-	
Industrial Links	-	

Non-Technical Abstract: Alginate is a biocompatible and biodegradable anionic polysaccharide with high application potential due to its reactivity and selectivity towards various metal cations. These latter interact with the functional groups on the polymer backbone, resulting in crosslinked hydrogels with characteristic porosities and mechanical properties. The goal of the experiment is to investigate internal structure of freeze-dried alginate fibers crosslinked with different cations by means of scanning electron microscopy (SEM). SEM already demonstrated its potential for the observation of the morphology of the fibers at the nano and micro-scale, but the imaging of the internal structure of the fibers is not straightforward, as a conventional cutting procedure would likely induce modifications and, as a consequence, the observation of artifacts.

Publications

ISIS neutron and muon source		**IM@IT E-platform:** Yes - ISIS Facility
Instruments	**IMAT**	**Days Requested**
Access Route	Direct Access	**Previous RB Number:**
Science Areas		
Sponsored Grant	None	**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 **FIB-SEM GAIA 3** **Days Requested:** 1
Special requirements:

SAMPLE

Material	Alginate xerogel crosslinked with Cu(II) ions	Alginate xerogel crosslinked with Ca(II) ions	Alginate xerogel crosslinked with Mn(II) ions
Formula	(C6H8O6)n(Cu)m	(C6H8O6)n(Ca)m	(C6H8O6)n(Mn)m
Forms	Solid	Solid	Solid
Volume	cc	cc	cc
Weight	100 mg	100 mg	100 mg
Container or substrate	The xerogels can be directly fixed on the FIB-SEM support	The xerogels can be directly fixed on the FIB-SEM support	The xerogels can be directly fixed on the FIB-SEM support
Storage Requirements	-	-	-

SAMPLE ENVIROMENT

Temperature Range	Room temperature - K	Room temperature - K	Room temperature -
Pressure Range	- - mbar	- - mbar	- - mbar
Magnetic field range	- - T	- - T	- -
Standard equipment	None	None	None
Special equipment	No special equipment	No special equipment	No special equipment

SAFETY

Prep lab needed	No	No	No
Sample Prep Hazards	No	No	No
Special equip. reqs	No special equipment required	No special equipment required	No special equipment required
Sensitivity to air	No	No	No
Sensitivity to vapour	No	No	No
Experiment Hazards	No	No	No
Equipment Hazards	-	-	-
Biological hazards	No	No	No
Radioactive Hazards	No	No	No
Additional Hazards	-	-	-
Additional Details	-	-	-
Sample will be	Disposed of by instrument scientist	Disposed of by instrument scientist	Disposed of by instrument scientist

Science Case

Study of the internal structure of alginate fibers crosslinked with different cations by means of FIB-SEM

1. Background and Context

Alginate is a biocompatible and biodegradable anionic polysaccharide with high application potential due to its reactivity and selectivity towards various metal cations. These latter interact both with the hydroxyl and carboxylate groups on the polymer backbone, resulting in crosslinked hydrogel structures with characteristic porosities and mechanical properties. In order to foster the use of biopolymeric fabrics, we profitably used an extrusion and crosslinking approach (also known as wet spinning) for the preparation of metal-alginate fibers. The optimization of multiple parameters, *i.e.,* flow of extruded polymer, concentration of the polymeric solution, and crosslinker concentration, makes it possible to easily produce filiform structures with lengths up to some meters. Furthermore, the type of cation and the related complexation geometries allow one to obtain products suitable for several applications (wound healing, water purification, flame retardancy etc.). Fibers from different cations also proved to possess different internal morphology, as a result of the specific diffusion and crosslinking process. These aspects of the fiber-formation process 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 Ph.D. student in co-tutorship between the University of Florence (Italy) and the University of Strasbourg (France), started on November 1st 2022 and funded by the Italian Ministry of University and Research (MUR) for three years. The project aims to realize alginate-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), specifically in the Nanochemistry Lab of Professor Paolo Samorì. The Lab is an excellence in the preparation of 0D to 3D multicomponent nanostructures and networks for energy, sensing and optoelectronic applications. An accurate description of the self-assembly properties of alginate in the presence of cations will be useful for the preparation of 2D alginate-graphene pressure-sensitive devices.

2. Proposed experiment

The aim of the experiment is to observe the internal morphology of freeze-dried alginate fibers crosslinked with various cations (Cu^{2+}, Ca^{2+}, Mn^{2+} and Ag^{+}) by means of Focused Ion Beam - Scanning Electron Microscopy (FIB-SEM). Different cations induce a different structural organization of alginate chains, due to their different size/charge/coordination geometry. This process results in alginate hydrogel fibers with distinct physico-chemical properties. The characterization of the internal structure of the fiber 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. In order to expose the internal cross-section of the fiber without altering it during the cutting process, FIB offers a unique opportunity. The use of ultramicrotomy, also available within the facility, will be evaluated as an alternative method to obtain a reliable cross-section of the fiber. Chemical staining with, for instance, uranyl acetate, will also be considered to improve the contrast of the xerogel matrix during the imaging. The imaging of the obtained inner structure with SEM will allow one to obtain micrographs that will give information on the type of internal structure of the fibers in terms of pore size and geometry. The obtained results will be complementary to neutron tomography experiments on the swollen fibers that might be carried out at ISIS facility (IMAT instrument, proposal to be sent during the next round). Such experiments would allow for the 3D reconstruction of the fiber internal structure when swollen in water, providing a useful comparison to the structure of the freeze-dried fibers obtained by means of FIB-SEM.

3. Summary of previous experimental proposals or characterisation

The extrusion and crosslinking process already highlighted differences between the fibers prepared with different cations, in terms of density and external morphology (see **Figure 1a** and **1b**). Differences in internal morphology between swollen fibers were also evident from the optical analysis of their cross-sections (**Figure 1c**).

FT-IR spectroscopy was also used to determine the complexation geometries of the alginate complexes with different cations, due to the high sensitivity of the carboxylate groups to coordination that results in shifts of the asymmetric stretching vibration band (**Figure 1d**). Specifically, the Cu^{2+}-alginate and Ag^{+}-alginate complexes highlighted a *pseudo-bridging unidentate* and a *bidentate bridging* coordination modes, respectively. This result also contributes to pointing out that different cations impart a different structure to alginate fibers, suggesting that differences in their internal structure might be present. SEM proved to be useful to characterize the morphology of the various crosslinked alginate samples (**Figure 1e** and **1f**), but the obtained information was limited to their external surface due to the lack of a reliable strategy to cut the samples and evaluate the morphology of the cross-sections.

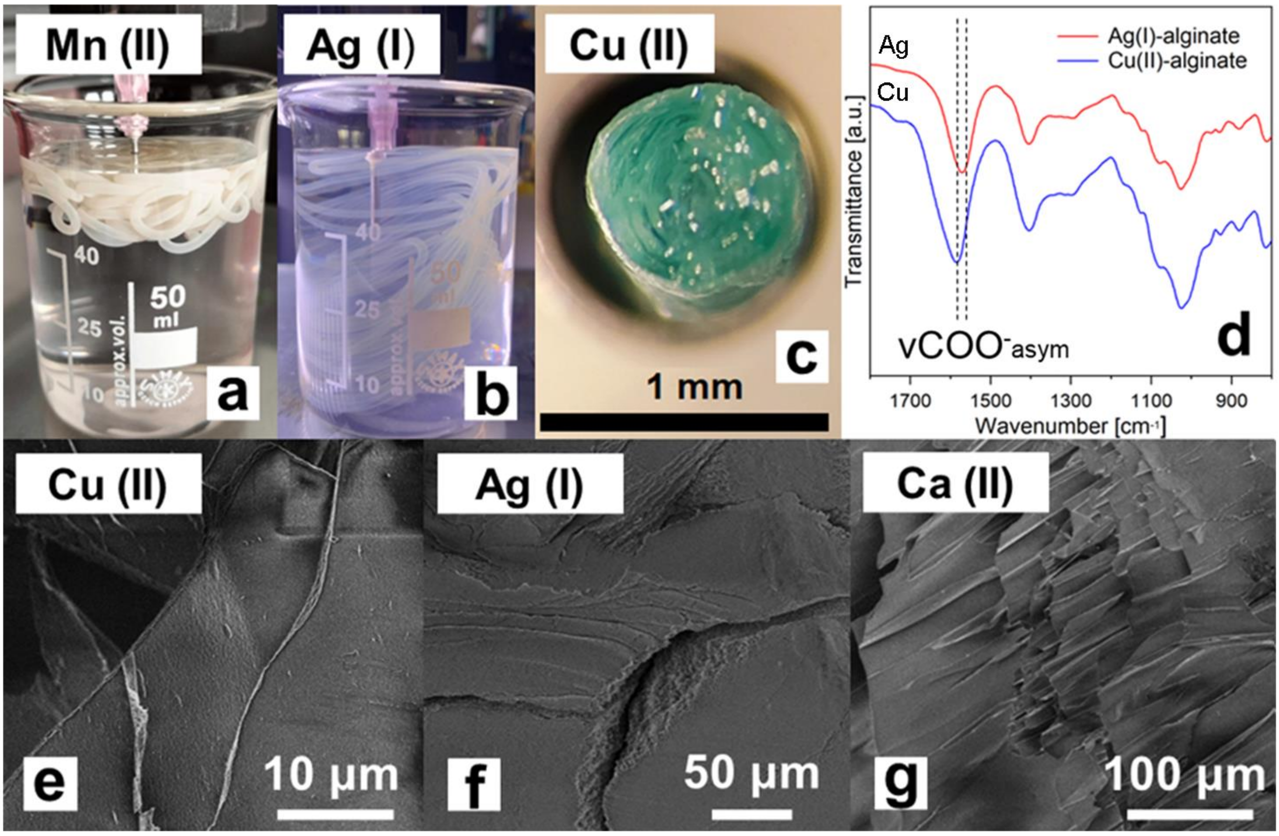

Figure 1. Extrusion and crosslinking of (a) Mn(II)-alginate and (b) Ag(I)-alginate fibers. (c) Swollen Cu(II)-alginate fiber's cross-section. (d) FT-IR spectra of Ag(I)-alginate and Cu(II)-alginate. (e) Cu(II)-alginate, (f) Ag(I)-alginate and (g) Ca(II)-alginate SEM micrographs of the external portion of the fiber.

4. Justification of experimental proposals request

FIB-SEM TESCAN GAIA 3 was selected for this experiment as it allows for the imaging of fibers' cross-sections: the ion beam can be focused on the sample to precisely cut a cross-section, so to expose the internal structure for the imaging with electron microscopy. The samples that will be analyzed consist of four freeze-dried fibers, made of alginate hydrogels crosslinked with Cu^{2+}, Ca^{2+}, Mn^{2+} and Ag^{+}. To carry out the procedure of the fibers with the FIB and their imaging with SEM, a total of 1 day of operation is requested.