

# Experiment Proposal

Experiment number GP2023039

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**Experiment title**

Semi-interpenetrated PEG-based hydrogels as transparent network with pH-responsive porosity for diffusivity studies: structural and morphological investigation

**MRF Instrument**
**SAXS Xenocs Xeuss**
**Days requested:** 3

**Access Route**

Direct Access

**Previous GP Number:** No

**Science Areas**

Chemistry

**DOI:** -

**Sponsored Grant**

None

**Sponsor:** -

**Grant Title**

-

**Grant Number:** -

**Start Date**

-

**Finish Date:** -

**Similar Submission?**

-

**Industrial Links**

-

**Non-Technical Abstract**

Hydrogels of polyethylene glycol (PEG) represent a widely used biocompatible and hydrophilic alternative in the development of biomedical applications. Conventional polymeric porous matrices require the addition of porogen agents. We report an approach to develop transparent biocompatible PEG with tunable pH-responsive porosities in the micron-scale. Preliminary tests showed the responsiveness introduced by adding poly (acrylic acid) to the PEG polymeric network and suggested significant structural changes from nanoscale topology to micron-scale morphology. A morphological characterization of so-prepared 3D heterogeneous porous matrices could allow a better understanding of diffusive properties of colloids and complex living systems (i.e., bacteria) in addition to the relevance that smart hydrogels have in several research fields as cell growth or adhesion, filtration, and drug delivery.

**Publications**

-

**ISIS neutron and muon source**
**IM@IT 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 Gavino Bassu, Università degli studi di Firenze, ITALY  
**MRF Instrument** **SAXS Xenocs Xeuss** **Days Requested: 3**  
**Special requirements:**

### SAMPLE

<b>Material</b>	PEG/PAA: Polyethylene glycol hydrogels (PEG) with interpenetrated poly (acrylic acid) (PAA)	-	-
<b>Formula</b>	PEG: $C_{2n}H_{4n+2}O_{n+1}$ Mw = 35 kDa; PAA: $(C_3H_4O_2)_n$ Mw = 420 kDa	-	-
<b>Forms</b>	Solid		
<b>Volume</b>	1 cc		
<b>Weight</b>	1 mg		
<b>Container or substrate</b>	Sample holder for Gel	-	-
<b>Storage Requirements</b>	-	-	-

### SAMPLE ENVIROMENT

<b>Temperature Range</b>	288,15 - 298,15 K	-	-
<b>Pressure Range</b>	1013,25 - N.A. mbar	-	-
<b>Magnetic field range</b>	N.A. - N.A. T	-	-
<b>Standard equipment</b>	None	-	-
<b>Special equipment</b>	No special equipment are requested	-	-

### SAFETY

<b>Prep lab needed</b>	Yes	-	-
<b>Sample Prep Hazards</b>	No other hazards are associated with the sample	-	-
<b>Special equip. reqs</b>	No otherspecial equipment are requirements	-	-
<b>Sensitivity to air</b>	No	-	-
<b>Sensitivity to vapour</b>	No	-	-
<b>Experiment Hazards</b>	No other hazards are associated with the experiment	-	-
<b>Equipment Hazards</b>	-	-	-
<b>Biological hazards</b>	No Biological hazards are associated with the sample	-	-
<b>Radioactive Hazards</b>	No Radioactive hazards are associated with the sample	-	-
<b>Additional Hazards</b>	-	-	-
<b>Additional Details</b>	-	-	-
<b>Sample will be</b>	Disposed of by instrument scientist	-	-



## **Semi-interpenetrated PEG-based hydrogels as transparent network with pH-responsive porosity for diffusivity studies: structural and morphological investigation**

### **1. Background and Context**

Hydrogels of polyethylene glycol (PEG) represent a widely used biocompatible and hydrophilic alternative in the development of biomedical applications. Conventional polymeric porous matrices require the addition of porogen agents whose nature could limit their application and strictly determines their morphology. An interesting alternative approach is the freeze-thaw (FT) treatment of the previously formed gel; the porosity is subsequently induced by the ice-crystals those represent the porogen agent. Thanks to this method it is possible to induce the desired micron-scale porosity to the polymeric matrix by the nucleation and growth of the ice-crystals inside the hydrogel without any additional agent. The so-reported method allowed the development of biocompatible polymeric matrices with a high transparency degree and tunable porosities based on the monomer content in the pre-reaction mixture<sup>1</sup>. The presented method can be easily implemented in the development of smart hydrogels by the introduction of stimuli-responsive semi-interpenetrated polymers allowing responsive structural changes in the final polymeric network. Here we applied this approach to develop transparent biocompatible hydrogels with micron-scale porosities responsive to the pH environment. As shown in fig. 1-1, the addition of poly (acrylic acid) (PAA) in the pre-reaction mixture did not significantly affect the transparency degree of the gel before nor after the FT treatment. The precise morphological characterization of so prepared 3D heterogeneous porous matrices, well representative of the natural porous environment, could allow a better understanding of diffusive properties of colloids and complex living systems (i.e., bacteria)<sup>2</sup> in addition to the great relevance that smart hydrogels have in several research fields as cell growth or adhesion, filtration, and drug delivery.

### **2. Proposed experiment**

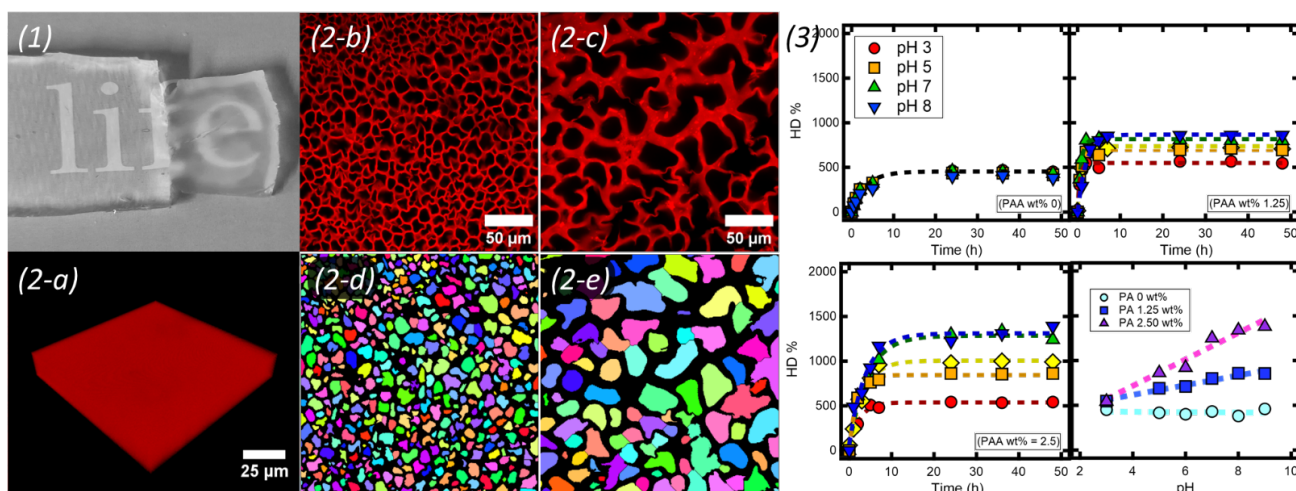
Experimental evidences have shown that transport properties of colloids and active living systems are strictly related within the matrix's morphology and can strongly deviate from the unconfined diffusive models. Such properties are related to pores size and morphology, which can be tailored by varying the monomer content in the pre-reaction mixture or by the PAA content and the pH. Some of the PEG/PAA hydrogels with tailored pores have been preliminary characterized through Confocal Microscopy (CLSM) to test the responsivity to pH changes. However, a deep morphological and geometrical characterization of volumetric stacks is required to completely understand the structural changes introduced by PAA at the different dissociation degrees imposed by the pH changes. Confocal Microscope 1: Leica TCS SP8 can support the specific micron-scale characterization of the developed systems in controlled hydration conditions. The volumetric stacks will be then processed through MorphoLibJ<sup>3</sup>, a mathematical library of ImageJ<sup>4</sup>, to reconstruct the 3D porosity and extrapolate the desired structural features. Coupled to CLSM a complete overview of connectivity, geometry, and symmetry of sub-micron porosities will be accessed via the high-resolution 3D reconstructions of FIB-SEM. The nanoscale investigation by U-/SAXS analysis through the Xeuss 3.0 HR apparatus are also requested to extrapolate information on the nanoscale topology and how PAA presence affect the polymeric structure in response to the different pHs. Thanks to this investigation, the average mesh-size and dimension of solid-like inhomogeneities ranging from few nm up to the micron scale could be explored; the general model of Debye-Bueche<sup>5</sup> allows the extrapolation of these information as well as the Porod exponent and the relative intensities ratio ( $I_{\text{lor}}(0)/I_{\text{ex}}(0)$ ), those reflecting the local compactness and the relative abundance of the scattering from the polymer network meshes with respect to the solid-like inhomogeneities.



### 3. Summary of previous experimental proposals or characterisation

In previous experiments, we investigated the swelling behaviour of PEG/PAA hydrogels with different content of the pH-responsive component, specifically 0, 1.25, and 2.5 wt%. The PAA addition did not significantly alter the equilibrium water content of PEG hydrogels ( $\approx 90\%$ ) but successfully introduces the desired pH-responsivity as demonstrated by the hydration degree (HD) kinetics in fig. 1-3. Consistently with the expectation, the equilibrium hydration degree of PAA-interpenetrated networks follows a linear increase with the increase of the pH value while the conventional PEG hydrogel kept the HD constant in the whole investigated pH range. In perfect agreement with the well-known physicochemical properties of the PAA, the acid groups are predominantly undissociated below  $\text{pH} = 3$ , where the HD converges to the conventional PEG gels; while, increasing the pH, the PAA-semi-interpenetrated gels start to diverge with HD values consistently proportional to the PAA content in the pre-reaction mixture (1.25 and 2.50 wt%). After the complete dissociation of the acid groups, the PEG/PAA gels exposed no significant changes in the HD over the pH 8, suggesting the complete swelling of the polymeric network.

Preliminary CLSM confirmed the absence of micron-scale inhomogeneities before the FT treatment and highlighted the structural changes of the pH-responsive systems (see fig 1-2). As suggested from the HD the polymeric network expose smaller porosities at pH 3 than the same system at pH 7 (panel 2-b and c, respectively). The images were processed by the mathematical library MorphoLibJ from ImageJ<sup>3,4</sup> revealing at pH 3 and 7, average pore diameters of 6.8 and 18.7  $\mu\text{m}$  and pore area fractions of 40 and 50 %, respectively.



**Figure 1.** PEG/PAA gels with 2.50 wt% of PAA before and after the FT treatment (1). Confocal images of gel before FT (2-a) and after the FT at pH 3 and 7 (2-b and c) and corresponding porosities highlighted by MorphoLibJ (2-d and e). HD profiles of PEG/PAA gels with 0, 1.25 and 2.50 wt% of PAA as a function of time, and the equilibrium hydration degrees as a function of pH.

