Supplementary Information

Engineering of Green Sterilization Technology to Obtain Biocompatible Aerogels: Supercritical CO_2 versus Ethylene Oxide and Gamma Radiation

María Carracedo-Pérez[1], Antonella Caterina Boccia[2], Inés Ardao[3], Cláudia P. Passos[4], Víctor Santos-Rosales[1], Beatriz Santos[5], Fabio Bernardo[6], María Blanco-Vales[1], Beatriz Magariños[5], Carlos A. García-González[1,*]

[1]AerogelsLab, I+D Farma Group (GI-1645), Department of Pharmacology, Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, iMATUS and Health Research Institute of Compostela E-15782, Spain

[2]Institute of Chemical Sciences and Technologies-SCITEC "G. Natta", CNR-National Research Council, Via A. Corti, 12, 20133 Milano, Italy

[3]BioFarma Research Group, Department of Pharmacology, Pharmacy and Pharmaceutical Technology, Innopharma Drug Screening and Pharmacogenomics Platform, Centro Singular de Investigación en Medicina Molecular y Enfermedades Crónicas (CiMUS), Universidade de Santiago de Compostela, Santiago de Compostela E-15782, Spain

[4] LAQV-REQUIMTE, Department of Chemistry, Universidade de Aveiro, 3810-193 Aveiro, Portugal

[5]Department of Microbiology and Parasitology, Aquatic One Health Research Center (iARCUS), Faculty of Biology, CIBUS, Universidade de Santiago de Compostela, Santiago de Compostela 15782, Spain

[6]CICECO, Department of Chemistry, Universidade de Aveiro, 3810-193 Aveiro, Portugal

*Corresponding Author: carlos.garcia@usc.es

SI 1. Materials and methods

SI 1.1. Proton Quantitative Nuclear Magnetic Resonance (^{1}H q NMR) spectroscopy

All starch samples were prepared by dissolving about 10 mg of aerogel in 750 µL of deuterated dimethylsulfoxide-d6 (d-DMSO), at 320K; spectra were referenced to the residual solvent peak at 2.50 ppm. Quantitative acquisition parameters for ^{1}H spectra: 90° pulse as calculated for each sample; relaxation delay 40.0 s. Spectral width 9615 Hz; number of transient 128, 90° pulse 7.93 µs PL1 −11.48 dB. The proton spin-lattice relaxation time (T1) of 40s was considered longer enough to ensure accurate quantification results, according to literature data. Data processing: exponential line broadening of 0.1 Hz was applied as resolution enhancement function; zero-filling to 32 K prior FT. Acquisition parameters for ^{1}H spectra of alginate aerogels in D_2O: 90° pulse 8.24 µs PL1 −11.48 dB; relaxation delay 2.0 s. Spectral width 10869 Hz; number of transient 128. For the quantification of H_2O_2 entrapped within the alginate matrix aerogels, a defined amount of aerogel was incubated in d-DMSO at 40°C for 30 minutes, then the supernatant was immediately transferred in an NMR tube for data acquisition. This procedure differed from that used for starch-based aerogels due to the limited solubility of alginate in DMSO.

The H_2O_2 on the $scCO_2$ sterilized samples were quantified using both external and an internal standard. The external standard consisted of a solution with a known concentration of H_2O_2 in d-DMSO. A 0.153 mol·L^{-1} stock solution was prepared by dissolving 10 µL of H_2O_2 solution in 0.75 mL of d-DMSO, and subsequent external standard solutions were obtained by serial dilution. The concentration of the external standard solutions is listed in Table S1. Among them, solution six, with a concentration of 6.13 mM/L, was selected as the external standard to determine the concentration of H_2O_2 in the aerogel samples and to verify the concentration of the prepared solutions, thereby validating the protocol. The results demonstrated excellent agreement between the prepared and experimentally measured concentrations except for solution 4, which represents the detection limit.

Table S1. H_2O_2 concentration as prepared and determined by qNMR

Solution	Prepared [mmol/L]	Experimental [mmol/L]*
1	0.078	0.079
2	1.55	1.60
3	76.50	74.21
4	$4.6\ 10^{-5}$	$2.05\ 10^{-5}$
5	38.25	38.28
6	6.13	
7	14.28	14.77

*Calculated with respect to solution 6, selected as external standard solution

The model was validated using a toluene solution of known molar concentration, (6.27 mmol/L), as internal standard, following the ^{1}H qNMR protocol on a set of randomly selected samples.

SI 2. Results

SI 2.1. H_2O_2 concentration quantification by ^{1}H q NMR

The results in table S2 demonstrated good agreement, confirming the reliability of the model.

Table S2. Comparison of H_2O_2 concentration by using an external and an internal standard. Conditions in bold text correspond to the selected optimized conditions.

Sample	External standard ppm	Internal standard ppm
STA_scCO$_2$Co	183	182
STA_scCO$_2$Co_V2	509	-
STA_scCO$_2$Co_V24	**132**	**131.7**
STA_scCO$_2$Co_2.5bar	178	177.5
STA_scCO$_2$Co_50bar	238	-

The external standard is a solution of [H_2O_2] = 6.13 mmol/L. The internal standard is a toluene solution in d-DMSO

SI.2.2. Gel permeation chromatography of sterilized aerogel samples

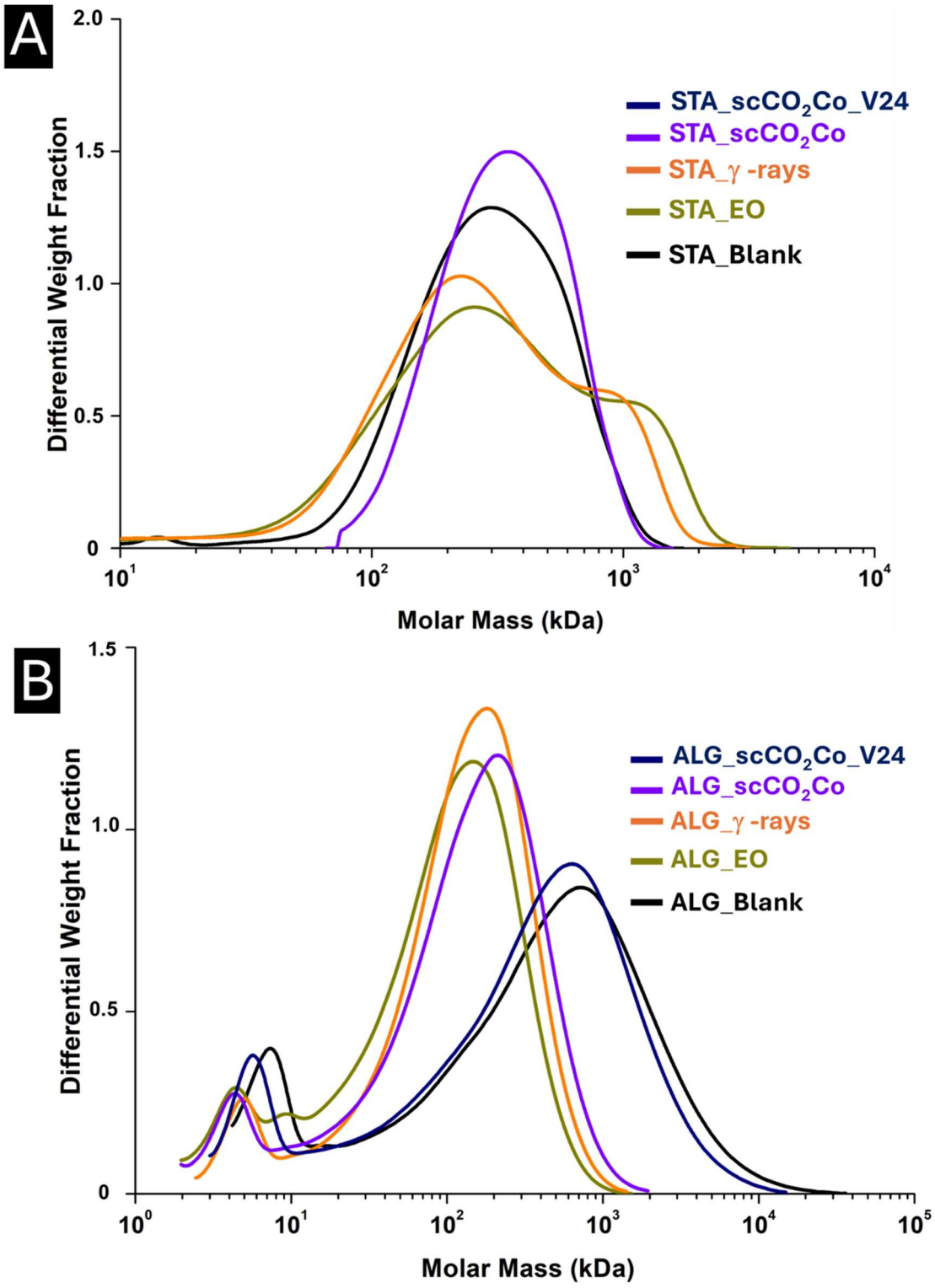

Figure SI.1. Gel permeation chromatography molecular weight distribution of sterilized (A) starch samples and (B) alginate samples