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► **To cite this version:**

Caroline Jain-Beuguel, Xue Li, Ludivine Houel-Renault, Tina Modjinou, Christelle Simon-Colin, et al.. Water-Soluble Poly(3-hydroxyalkanoate) Sulfonate: Versatile Biomaterials Used as Coatings for Highly Porous Nano-Metal Organic Framework. *Biomacromolecules*, 2019, 20 (9), pp.3324-3332. 10.1021/acs.biomac.9b00870 . hal-02913754

HAL Id: hal-02913754

<https://hal.science/hal-02913754>

Submitted on 31 Jan 2024

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Water-soluble poly(3-hydroxyalkanoate) sulfonate: versatile biomaterials used as coatings for highly porous nano-MOF

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Abstract :

Water soluble poly(3-hydroxyalkanoate) containing ionic groups was designed by two successive photoactivated thiol-ene reactions. Sodium-3-mercaptopropionate (SO₃⁻) and poly(ethylene glycol) methyl ether thiol (PEG) were grafted onto poly(3-hydroxyoctanoate-co-3-hydroxyundecenoate) PHO(67)U(33) to both introduce ionic groups and hydrophilic moieties. The grafted copolymers PHO(67)SO₃-(20)PEG(13) were then used as biocompatible coatings of nano-Metal Organic Frameworks surface. Scanning Electron Microscopy and Scanning Transmission Electron Microscopy coupled with Energy Dispersive X-ray characterizations have clearly demonstrated the presence of the copolymer on the MOF surface. These coated nano-MOF are stable in aqueous and physiological fluids. Cell proliferation and cytotoxicity tests performed on murine macrophages J774.A1 revealed no cytotoxic side effect. Thus, biocompatibility and stability of these novel hybrid porous MOF structures encourage their use in the development of effective therapeutic nanoparticles.

Keywords : Polyhydroxyalkanoates, PHOU, thiol-ene photoactivated, PEG, MOF, nanoparticles

1. INTRODUCTION

Poly(3-hydroxyalkanoates) (PHAs) are biodegradable polymers synthesized by many organisms, especially prokaryotes. There are over 150 types of these polyesters, accumulated as carbon and energy storage material in a wide variety of bacteria. PHA granules are produced when microorganisms are cultivated in the presence of both carbon source excess and nitrogenous nutrient deficiency.¹⁻² According to the length of side chains, different types of PHAs can be classified as either a short chain length, scl-PHAs, a medium chain length, mcl-PHAs and lcl-PHA, long chain length. Physical properties are related to the length of these side chain; thus, scl-PHAs are semi-crystalline, rigid and brittle whereas mcl-PHAs are soft and elastomeric.

Over the last three decades, biomaterials based on PHAs have been developed for medical or pharmaceutical applications³⁻¹² such as tissue engineering, implants or drug delivery carriers due to their proven biodegradability, biocompatibility and lack of toxicity. In order to enlarge the potential in biomedical applications, many chemical modifications have been developed. Among them, the combination of the properties of hydrophobic PHA and hydrophilic PEG was used to prepare amphiphilic PHAs. The excellent water-solubility and biocompatibility of PEG, encouraging the use of this polymer in biomedical applications.¹³⁻¹⁵ Moreover PEG having molar mass in the range of 2,000-5,000 g/mole confers a long blood-circulation ability by reducing both nanoparticles accumulation in liver and spleen, and mononuclear phagocyte system uptake (stealth behaviour),¹⁶ in order to avoid *in vivo* nanoparticles rapid elimination. Micro- and nanoparticles based on amphiphilic PHA-block-PEG copolymer have been extensively studied as drug delivery system.¹⁷⁻²⁸ PEGylation of PHAs can be performed by chemical modifications or by bioprocessing *in vivo*, bioPEGylation. Regarding the chemical way, block copolymers were synthesized by direct transesterification or by using telechelic oligomers based on PHAs. A series of amphiphilic diblock and graft PEG-PHA copolymers were recently prepared through a click chemistry approach.²¹⁻²² Another way to improve hydrophilicity or water-solubility of organic compound is to introduce ionic groups as carboxylic groups,²⁹ ammonium groups,³⁰ N-acetyl-L-lysine³¹ and sulfonate functions.³² The sulfonate anionic sites are known to display biological interactions and they can interact with proteins.³³ A series of PHAs sulfonate have been prepared using the highly versatile approach as photoactivated thiol-ene or thiol-yne reactions. These new amphiphilic scl-PHAs oligomers were able to self-assemble in aqueous solution whereas the sulfonate derivatives of mcl-PHAs are totally soluble in water if the content of sulfonate groups is superior to 25% whatever the molar mass of the PHAs.³² In this context, we used here the large versatility of the thiol-ene

1
2
3 72 reaction to introduce both sulfonate groups and PEG grafts. It has already been demonstrated
4
5 73 that the PHAs having unsaturated groups in the side chains are very useful to introduce reactive
6
7 74 groups by using thiol-ene reactions. Poly(3-hydroxyoctanoate-co-3-hydroxyundecenoate)
8
9 75 PHO₍₆₇₎U₍₃₃₎ is a functionalized mcl-PHA bearing molar ratio 33% of terminal alkene units on
10
11 76 its side chains. First, thiol containing sulfonate groups were reacted with unsaturated PHAs. In
12
13 77 a second step, the PEG grafting was achieved *via* a similar thiol-ene addition, which has proven
14
15 78 to be an effective reaction to chemical modify the unsaturated PHAs.^{22,31,34}
16
17 79 The objective here was to propose a versatile biodegradable and biocompatible polymeric
18
19 80 coating for highly porous nanosized metal organic frameworks (nanoMOFs). NanoMOFs,
20
21 81 formed by strong coordination between metal ions and organic ligands, recently emerged in the
22
23 82 world of therapeutic nanovectors.³⁵⁻³⁹ In particular, iron trimesate nanoMOFs MIL-100(Fe)
24
25 83 (MIL stands for Material from Institut Lavoisier) have large surface areas allowing to load a
26
27 84 large variety of drugs able to penetrate within the porous MOF structures.⁴⁰⁻⁴¹ These porous
28
29 85 solids are built up from Fe(III) octahedra trimers and trimesate linkers (1,3,5-benzene
30
31 86 tricarboxylate) that self-assemble to build a porous architecture delimiting large (29 Å) and
32
33 87 small (24 Å) mesoporous cages. The two types of cages are accessible for drug adsorption inside
34
35 88 the 3D-porosity through pentagonal (5.6 Å) and hexagonal windows (8.6 Å). Due to the
36
37 89 presence of Fe and free water molecules in their structure, they acted as efficient T₂-weighted
38
39 90 contrast agents for Magnetic Resonance Imaging (MRI) of interest for theranostics.⁴² The lack
40
41 91 of toxicity of MIL-100(Fe) nanoMOFs has been established *in vivo* after repetitive
42
43 92 administrations.⁴³⁻⁴⁵ These materials offer a particular hydrophilic/hydrophobic
44
45 93 microenvironment inside their open porosity, allowing the incorporation of a large variety of
46
47 94 either water-soluble or hydrophobic active molecules, by simple one-step impregnation,
48
49 95 generally without organic solvent.⁴¹⁻⁴²
50
51 96 For last five years, there has been an intensive research in coating nanoMOFs in order to prevent
52
53 97 premature drug release, improve colloidal stability, and control the *in vivo* fate. For instance,
54
55 98 oligomers or polysaccharides (cyclodextrin, heparin, hyaluronic acid or chitosan),^{43,44,46-49}
56
57 99 dioleoyl-phosphocholine lipid⁵⁰ or polycaprolacton⁵¹ were used. Of main interest, PEG grafting
58
59 100 onto MOF nanoparticles surface was also performed to generate stealth material against
60
61 101 immune system.^{43,52-54} We here developed a new hybrid system, composed of nanoporous
62
63 102 MIL-100(Fe) nanoMOF coated by a water-soluble PEGylated PHA containing sulfonate groups
64
65 103 that should be able to strongly interact with nanoMOF. Thus, all or part of PHOU lateral
66
67 104 unsaturations were first functionalized with SO₃⁻ and PEG groups, in order to improve water-
68
69 105 solubility and achieve efficient PEG grafting as well as metal interactions between

1
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3 106 functionalized PHA and MOF. Then, MOF-PHA hybrid nanoparticles were synthesized by a
4
5 107 rapid one-step non-covalent method or impregnation, and their structure was investigated by
6
7 108 complementary physicochemical methods. More particularly, for the first time, scanning
8
9 109 transmission electron microscopy (SEM-STEM) using energy dispersive X-ray (EDX)
10
11 110 spectroscopy was used to unravel the structure of the coated nanoMOFs and enable gaining
12
13 111 information on the coating location. Cell proliferation and cytotoxicity tests were carried out
14
15 112 on murine J774.A1 macrophages to evaluate MOF-PHA hybrid porous nanoparticles toxicity.
16
17 113

18 114 2. MATERIALS AND METHODS

19 115 20 116 2.1. Materials

21
22 117 Dimethyl sulfoxide (DMSO), methanol (MeOH) and tetrahydrofuran (THF) were obtained
23
24 118 from VWR International. 2,2-dimethoxy-2-phenylacetophenone (DMPA), sodium-3-mercapto-
25
26 119 1-ethanesulfonate (SO_3^-), poly(ethylene glycol) methyl ether thiol (PEG, molar mass: 2,000
27
28 120 $\text{g}\cdot\text{mol}^{-1}$) were purchased from Sigma Aldrich. Dialysis membranes (MWCO 1 and 6-8 KDa)
29
30 121 were procured from Spectrumlabs. Poly(3-hydroxyoctanoate-co-3-hydroxyundecenoate)
31
32 122 $\text{PHO}_{(67)}\text{U}_{(33)}$ was provided from HES-SO Valais-Wallis, Switzerland.
33
34 123

35 124 2.2. Synthesis of $\text{PHO}_{(67)}\text{U}_{(13)}\text{SO}_3^-(20)$

36
37 125 100 mg of $\text{PHO}_{(67)}\text{U}_{(33)}$ ($M_n = 40,000 \text{ g/mol}$, $2.13 \times 10^{-4} \text{ mol C=C}$) and 0.2 eq of photoinitiator
38
39 126 DMPA/C=C were dissolved in 6 mL of THF (Solution 1). On the other hand, 1.2 eq of SO_3^-
40
41 127 /C=C were solubilized in 6 mL of MeOH then adding 3 mL of THF drop by drop (Solution 2).
42
43 128 Solution 2 was mixed with the first and the resulting mixture was irradiated during 10 min at
44
45 129 room temperature by Lightning cure LC8 (L8251, Hamamatsu) equipped with a mercury-xenon
46
47 130 lamp ($180 \text{ mW}\cdot\text{cm}^{-2}$) coupled with a flexible light guide whose end was placed 11 cm from the
48
49 131 sample. At last, 30 mL of DMSO were added to the resultant solution before purification by
50
51 132 dialysis (MWCO 1,000 Da) against 2 L of water for 2 days with 4 water changes per day,
52
53 133 followed by freeze-drying (yield 86%).
54
55 134

56 135 2.3. Synthesis of $\text{PHO}_{(67)}\text{U}_{(7)}\text{SO}_3^-(20)\text{PEG}_{(6)}$ and $\text{PHO}_{(67)}\text{SO}_3^-(20)\text{PEG}_{(13)}$

57 136 50 mg of $\text{PHO}_{(67)}\text{U}_{(13)}\text{SO}_3^-(20)$ were dissolved in DMSO at a concentration of 16.7 g/L before
58
59 137 adding 3 eq (for copolymer containing $\text{PEG}_{(6)}$) or 8 eq (for copolymer containing $\text{PEG}_{(13)}$) of
60
138 PEG/C=C and 0.2 eq of DMPA/C=C. The mixture was then irradiated during 30 min at a

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2
3 139 distance of 11 cm prior purification. Next, copolymer was diluted at 2 g/L in DMSO before
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5 140 dialysis against 2 L of water in 6-8 KDa MWCO membrane during 3 days with 4 water changes
6
7 141 per day, followed by freeze-drying. Manipulation was repeated 4 times until maximal
8
9 142 elimination of excess PEG (yields of 90 and 94%, respectively).

10 143

11 144 **2.4. Characterization**

12
13 145 ¹H NMR spectra were recorded in CDCl₃ or DMSO-d₆ on a Bruker AV400 MHz. Size
14
15 146 exclusion chromatography (SEC) experiments were determined in chloroform for PHOU using
16
17 147 Shimadzu LC-10AD pump with two Shodex GPC K-805L columns (5 μm Mixte-C) or in
18
19 148 LiNO₃⁻ 0.5 M solution for copolymers using two PL aquagel-OH 40-30 columns (8 μm Mixte-
20
21 149 C), both at a concentration of 10 mg/mL. A Wyatt Technology Optilab Rex interferometric
22
23 150 refractometer was used as detector, and low polydispersity index polystyrene standards (3 x10⁴
24
25 151 - 2 x10⁶ g/mol) for PHOU analysis, or PEO standards (polyethylene oxide, 106 - 4.6 x10⁴ g/mol)
26
27 152 for copolymers measurements, were used to calibrate the system. Differential scanning
28
29 153 calorimetry (DSC) measurements were performed on a Perkin Elmer Diamond DSC apparatus.
30
31 154 The following protocol was used for each sample: heating from -60 to +200 °C at 10 °C/min,
32
33 155 cooling to -60 °C at 200 °C/min, hold 5 min and heat again to 200 °C at 10 °C/min.

34
35 156 Transmittance measurements were performed on a Cary50 Bio Varian UV-Visible
36
37 157 spectrophotometer controlled by Cary Win UV software. The acquisition was performed from
38
39 158 250 to 800nm. A 1g.L⁻¹ solution of PHO₍₆₇₎U₍₇₎SO₃⁻(₂₀)PEG₍₆₎ in ultrapure water was used to
40
41 159 evaluate the solubility properties of the copolymer. The transmittance of this solution was
42
43 160 measured using ultrapure water as reference for the 100% of transmittance.

44 161

45 162 **2.5. Preparation of MOF-PHA nanoparticles**

46
47 163 MIL-100(Fe) iron-carboxylate nanoMOF were synthesized by microwave assisted
48
49 164 hydrothermal reaction as previously described.^{38,43} 1 mg (40 μL) of MOF was modified by
50
51 165 impregnation with PHO₍₆₇₎U₍₇₎SO₃⁻(₂₀)PEG₍₆₎ or PHO₍₆₇₎SO₃⁻(₂₀)PEG₍₁₃₎ (from 17 to 83% (w/w))
52
53 166 in a final MilliQ water volume of 500 μL (MOF final concentration 2 mg/mL) under gentle
54
55 167 rotative agitation for 1 h at room temperature in the dark.

56 168

57 169 **2.6. Characterization by STEM-EDX and TEM-EDX**

58
59 170 PHA-MOF nanoparticles size and surface charge were measured by dynamic light scattering
60
171 (DLS) and zeta potential (ZP) on a Malvern Zetasizer Nano ZS. PHA-MOF nanoparticles

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2
3 172 morphology was investigated by transmission electron microscopy (TEM) on a JEOL-2100F
4 173 microscope operating at 200 KV. Chemical composition was investigated with a FEI TECNAI
5 174 G2 F20 equipped with scanning TEM (STEM) device fitted to high angle annular dark field
6 175 imaging (HAADF), energy dispersive X-ray spectrometer (EDX) and electron energy-loss
7 176 spectrometer (EELS). Chemical composition was also performed with a Merlin (Carl Zeiss)
8 177 equipped with an EDX Advanced SDD X-Max detector (Oxford Instruments).
9
10
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15 179 **2.7. Cell culture**

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17 180 Murine macrophages J774.A1 adherent cells were grown at 37 °C under humidified atmosphere
18 181 with 5% CO₂ in Dulbecco's Modified Eagle Medium (DMEM) GlutaMAX™ supplemented
19 182 with 10% inactivated fetal bovine serum, penicillin G (100 U/mL) and streptomycin (0.1
20 183 mg/mL). During experiments, medium was exchanged three times per week.
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22
23
24

25 185 **2.8. Cytotoxicity assay**

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27 186 Into 96 well plate, J774.A1 were seeded at 20,000 cells/well and then 25, 50, 100, 200 or 400
28 187 µg/mL of uncoated MOF, copolymers of PHA grafted with 6 or 13% of PEG and MOF-PHA
29 188 nanoparticles were added following the manufacturer's protocols (LDH cytotoxicity assay,
30 189 Roche). After 24h incubation at 37 °C, absorbance was measured with a Clariostar microplate
31 190 reader (excitation at 490 nm and emission at 680 nm). As a positive control, cells were exposed
32 191 to medium containing sterile MilliQ water while untreated murine macrophages served as
33 192 negative control.
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39 193 40 41 194 **2.9. Cell proliferation assay**

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43 195 Into 96 wells plate, J774.A1 were seeded at 5,000 cells/well and then 25, 50 or 100 µg/mL of
44 196 uncoated MOF, copolymers of PHA grafted with 6 or 13% of PEG and MOF-PHA
45 197 nanoparticles were added following the manufacturer's protocols (RealTime-Glo™ MTT Cell
46 198 Viability Assay kit, Promega). After 72h incubation at 37 °C, bioluminescence intensity was
47 199 measured every day with a Glomax microplate reader. As a negative control, cells were exposed
48 200 to medium containing sterile MilliQ water while untreated murine macrophages served as
49 201 positive control.
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56 203 **3. RESULTS AND DISCUSSION**

204 **3.1. Preparation and characterization of poly(3-hydroxyalkanoate) sulfonate grafted with** 205 **PEG**

206 Poly(3-hydroxyoctanoate-co-3-hydroxyundecenoate) $\text{PHO}_{(67)}\text{U}_{(33)}$ is a hydrophobic polyester
207 composed of long alkyl side chains and lateral alkene groups. The interest in preparing PHA-
208 based copolymers that are both covalently grafted with PEG and sulfonate functions is twofold.
209 It allows both to bind it to the nano-MOF surface by ionic interactions and to avoid the
210 aggregation of nanoparticles due to hydrophilic PEG grafts whose modify the hydrodynamic
211 volume of particles. These particles also became less detectable by immune system cells. First,
212 sulfonate groups (SO_3^-) were grafted to the terminal double bonds of the $\text{PHO}_{(67)}\text{U}_{(33)}$, not only
213 to provide an amphiphilic character to the material,³² but also to induce non-covalent binding
214 of SO_3^- ions to Fe^{3+} ions that are present on the surface of MOF-type nanoparticles, by simple
215 impregnation as shown in Figure 1. $\text{PHO}_{(67)}\text{U}_{(33)}$ was characterized by ^1H NMR (Figure 2) to
216 determine the percentage of terminal unsaturations by integrating protons corresponding to the
217 CH peak (2) at 5.1 ppm, and the signal relating to the terminal alkene group of side chain (7) at
218 5.7 ppm. Sodium-3-mercapto-1-ethanesulfonate was grafted under photochemical activation,
219 using from 1 to 5 molar equivalents of sulfonates (SO_3^-). After purification by dialysis,
220 polymers were analyzed by ^1H NMR, and signals relating to the two CH_2 methylenes (c, d),
221 characteristic of sulfonate groups, appear at 2.66 ppm, indicating the success of the grafting.
222 The conversion percentages of the terminal double bonds to SO_3^- were calculated by comparing
223 the integrations of the protons corresponding to the CH peak (2) of the $\text{PHO}_{(67)}\text{U}_{(33)}$ structure at
224 5.1 ppm, with respect to the signal of terminal unsaturation HU side chain (7) at 5.7 ppm (Figure
225 3). The results obtained indicate a non-linear relationship between the conversion rate of double
226 bonds of $\text{PHO}_{(67)}\text{U}_{(33)}$ and the concentrations of sodium-3-mercapto-1-ethanesulfonate (RSH)
227 used. The copolymers were obtained with yields between 86 and 95%, reflecting the
228 effectiveness of the grafting method. In order to maintain a sufficient number of unsaturations
229 for the subsequent grafting of PEG methyl ether thiol, $\text{PHO}_{(67)}\text{U}_{(13)}\text{SO}_3^-(20)$ is therefore obtained
230 by using 1.2 equivalents $\text{RSH}/\text{C}=\text{C}$. The copolymer is not soluble in water, THF or even CHCl_3
231 as it is traditionally the case with native PHA whereas complete solubilization is observed in
232 DMSO.

233 In a second step PEG methyl ether thiol was grafted using the photoinduced thiol-ene reaction.
234 Low molecular weight PEG is a highly polar and water-soluble polymer, widely used in
235 pharmaceuticals for its biocompatibility and stealth properties. Two different ratios of PEG
236 methyl ether thiol (3 and 8 molar equivalents of PEG per mole of PHOU unsaturation) were
237 used (Figure 4). Two peaks are observed at 3.5 and 3.2 ppm, corresponding to the CH_2 (e) and

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3 238 CH₃ (f) methyl terminal groups of the PEG confirming the success of the grafting. The grafting
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5 239 percentages were calculated from the integrations of the signals of the PHOU at 5.1 ppm, and
6
7 240 the signal relating to the terminal unsaturations of the HU pattern side chain (7) at 5.7 ppm.
8
9 241 Two PEG ratios were found attesting the synthesis of copolymers of PHO₍₆₇₎U₍₇₎SO₃⁻(₂₀)PEG₍₆₎
10 242 with the use of 3 eq PEG/C=C, and PHO₍₆₇₎SO₃⁻(₂₀)PEG₍₁₃₎ in presence of 8 eq PEG/C=C with
11
12 243 a yield of 92%. The turbidity measurement showed a colorless solution at 550 nm indicating
13
14 244 absence of micelles or aggregates attesting of a total solubilization of HO₍₆₇₎U₍₇₎SO₃⁻(₂₀)PEG₍₆₎
15 245 and PHO₍₆₇₎SO₃⁻(₂₀)PEG₍₁₃₎ in water (Figure 5). PEG methyl ether thiol, PHO₍₆₇₎U₍₃₃₎ and the
16
17 246 copolymers of PHO₍₆₇₎U₍₇₎SO₃⁻(₂₀)PEG₍₆₎ and PHO₍₆₇₎SO₃⁻(₂₀)PEG₍₁₃₎ were analyzed by SEC
18
19 247 (Table 1). PEG methyl ether thiol has a molecular weight of 1,700 g.mol⁻¹ and native
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21 248 PHO₍₆₇₎U₍₃₃₎ has a molar mass of 40,000 g.mol⁻¹ with a polydispersity index of 1.7, a value
22
23 249 generally obtained is the case of natural PHAs. The copolymers of PHO₍₆₇₎U₍₇₎SO₃⁻(₂₀)PEG₍₆₎
24 250 and PHO₍₆₇₎SO₃⁻(₂₀)PEG₍₁₃₎ have molar masses of 24,000 and 29,500 g.mol⁻¹ respectively. The
25
26 251 difference of molar masses are explained by the difference of the chain conformations of
27
28 252 PHO₍₆₇₎U₍₃₃₎ and the grafted copolymers. Furthermore, the molar masses are expressed in PS
29 253 equivalent in the case of PHO₍₆₇₎U₍₃₃₎ and in PEG equivalents in the case of grafted copolymers
30
31 254 Consequently, the difference between natural PHO₍₆₇₎U₍₃₃₎ and grafted copolymers are due to
32
33 255 the difference of their solubility instead of a potential degradation of macromolecular chains. It
34
35 256 has been previously shown that thiol-ene reactions proceeded without any chain scission of
36
37 257 unsaturated PHAs.²⁰ DSC measurements confirm the semi-crystalline structure of the
38
39 258 copolymers (Figure 5). However, the values of the melting enthalpies of copolymers are lower
40
41 259 than that of the free PEG, thus showing some inhibitory action of PHA on the crystallization of
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43 260 PEG, a phenomenon already observed in similar work carried out by Babinot *et al.*²⁰ Similarly,
44
45 261 the presence of PEG polymers prevents the crystallization of PHA.

262 263 **3.2. Characterization of nano-hybrid MOF by electron microscopy and energy dispersive** 264 **X-ray spectrometer**

265 Transmission electron microscopy (TEM) images were performed on the native MOF and the
266 MOF-PHA hybrid nanoparticles, MOF-PHO₍₆₇₎U₍₇₎SO₃⁻(₂₀)PEG₍₆₎ and MOF-PHO₍₆₇₎SO₃⁻
267 (₂₀)PEG₍₁₃₎. The overlay of MOF-type nanoparticles by functionalized PHA does not impact
268 either the morphology or the size of the nanoparticles since average diameters between 250 and
269 300 nm have been measured (Figure 6). The proportions of C, O, Fe and S atoms were
270 quantified by STEM-EDX and SEM-EDX techniques. The presence of sulphur atoms,

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2
3 271 characteristic of the presence of functionalized PHA at the surface of the nanoMOF, was
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5 272 detected on the surface of both types of hybrid nanoparticles at equivalent atomic percentages
6
7 273 from both techniques (0.8%) (Table 2). The average S/Fe ratios were calculated in a very similar
8
9 274 way using the two STEM-EDX and SEM-EDX techniques (0.13-0.16), corresponding to one
10
11 275 sulphur atom as a marker for functionalized copolymers for eight iron atoms belonging to MOF.
12
13 276 These results confirm the adsorption of functionalized PHA copolymers on the surface of MOF-
14
15 277 type nanoparticles. The increase in the C/Fe ratio is all the more significant when the percentage
16
17 278 of PEG is high within copolymers, corresponding to rates of 11.6, 12.9 and 15.1 (STEM-EDX),
18
19 279 and 8.5, 10 and 11.1 (SEM-EDX), before and after coating nanoMOFs with $\text{PHO}_{(67)}\text{U}_{(7)}\text{SO}_3^-$
20
21 280 ${}_{(20)}\text{PEG}_{(6)}$ or $\text{PHO}_{(67)}\text{SO}_3^-{}_{(20)}\text{PEG}_{(13)}$ copolymers.

281

282 3.3. Stability of nano-hybrid MOF in aqueous medium

283 The two copolymers previously described were used to coat MOF nanoparticles to develop
284 stable porous MOF-PHA hybrid systems (Figure 1). The zeta potential measurements showed
285 a highly electropositive charge of +23 mV for native MOF nanoparticles, and electronegative
286 charges of -41 mV for $\text{PHO}_{(67)}\text{U}_{(7)}\text{SO}_3^-{}_{(20)}\text{PEG}_{(6)}$ and -24 mV for $\text{PHO}_{(67)}\text{SO}_3^-{}_{(20)}\text{PEG}_{(13)}$.
287 Different concentrations of $\text{PHO}_{(67)}\text{U}_{(7)}\text{SO}_3^-{}_{(20)}\text{PEG}_{(6)}$ and $\text{PHO}_{(67)}\text{SO}_3^-{}_{(20)}\text{PEG}_{(13)}$ from 0.4 to
288 10 g/L were placed in ultrapure water in presence of 2 g/L MOF. An instantaneous decrease in
289 zeta potential measured on the surface of MOF nanoparticles after contact with 1 g/L
290 copolymers was observed (Figure 7) thus confirming the coverage of MOF by functionalized
291 PHA copolymers. The diameter of the nanoparticles were measured by DLS (Figure 8). The
292 dispersion in water of the nanoMOF native or coated with 0.4 or 1 g/L PHA was not stable and
293 the particles agglomerated in 1 hour. Incubation of nanoMOF in the presence of low (0.4 or 1
294 g/L) concentrations of functionalized PHA was not sufficient to generate optimal recovery to
295 ensure the stability of nanoparticles over time. These results are in agreement with previous
296 studies showing the instability of the same nanoMOFs in aqueous media.⁴⁶ In contrast, the use
297 of 2 g/L of copolymers of $\text{PHO}_{(67)}\text{U}_{(7)}\text{SO}_3^-{}_{(20)}\text{PEG}_{(6)}$ and $\text{PHO}_{(67)}\text{SO}_3^-{}_{(20)}\text{PEG}_{(13)}$ successfully
298 allowed the development of stable hybrid nanoparticles. While $\text{MOF-PHO}_{(67)}\text{SO}_3^-{}_{(20)}\text{PEG}_{(13)}$
299 nanoparticles diameters remained stable for more than three days, $\text{MOF-PHO}_{(67)}\text{U}_{(7)}\text{SO}_3^-$
300 ${}_{(20)}\text{PEG}_{(6)}$ diameters measured by DLS were heterogeneous, greater than 350 nm during the first
301 48 hours, higher than the expected 270-300 nm. These data again reflect some aggregation of
302 particles possibly by a bridging effect and, in fact, insufficient coverage of PHAs on the surface
303 of MOFs to ensure steric stabilization. The best results in terms of stability in water were
304 obtained with the use of 4 g/L PHA regardless of the copolymer ($\text{PHO}_{(67)}\text{U}_{(7)}\text{SO}_3^-{}_{(20)}\text{PEG}_{(6)}$ or

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3 305 PHO₍₆₇₎SO₃⁻(₂₀)PEG₍₁₃₎) over a 72-hour period. Complementary experiments showed that MOF-
4 306 PHO₍₆₇₎SO₃⁻(₂₀)PEG₍₁₃₎ nanoparticles remained stable for more than 6 hours in PBS.
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8 308 **3.4. Biological tests**

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10 309 Grafted functionalized PHAs by using thiol-ene reaction did not demonstrate any significant
11 310 toxicity on the basis of viability assays on two different cell lines, mouse fibroblast cells
12 311 (NIH/3T3) and murine macrophage cells (J774.A1).⁵⁵ In this context, the possible *in vitro* toxic
13 312 effects of the MOF-PHA hybrid nanoparticles were determined using the LDH cytotoxicity.
14 313 The murine phagocytic cells J774.A1 were selected for these studies because they are model
15 314 cells, commonly used in cell biology and immunology, genetically stable and particularly
16 315 reactive in the presence of pathogens.⁵⁶ At concentrations lower than 50 µg/mL functionalized
17 316 PHA copolymers do not appear to generate toxic reaction against J774.A1 macrophages, as cell
18 317 viabilities close to 100% were observed (Figure 9). However, at concentrations in the range of
19 318 100-200 µg/mL, a proliferative effect was observed. This trend could be explained by processes
20 319 such as immune stimulation of the J774.A1 macrophages, which will have to be further
21 320 investigated. Finally, the use of hybrid nanoparticles MOF-PHO₍₆₇₎U₍₇₎SO₃⁻(₂₀)PEG₍₆₎ and
22 321 MOF-PHO₍₆₇₎SO₃⁻(₂₀)PEG₍₁₃₎ at concentrations between 25 and 100 µg/mL does not cause any
23 322 significant reaction from J774.A1 cells, since percentages of cell viability close to 100% were
24 323 observed. However, the administration of hybrid nanoparticles at higher doses results in a
25 324 decrease macrophage viability, ranging from 70 to 85% at 200 µg/mL. Thus, in view of further
26 325 *in vitro* investigations, the nanoMOF concentrations were limited to 100 µg/mL. The
27 326 proliferation of macrophage-type immune cells is a marker of phagocytic activation, a step
28 327 preceding the inflammatory response.⁵⁷⁻⁵⁸ The division of J774.A1 cells at 25, 50 or 100 µg/mL
29 328 of functionalized PHAs and native or hybrid MOF-type nanoparticles was measured over 42 h.
30 329 The cell proliferation experiments were conducted using the RealTime Glo kit which allows
31 330 the quantification of living cells from the bioluminescence emitted from the luciferase substrate,
32 331 reduced by the enzymatic activity of any viable cell. Results obtained after 42 h incubation of
33 332 J774.A1 macrophages with 25, 50 or 100 µg/mL of bare or hybrid PHA copolymers or
34 333 nanoMOFs are shown in Figure 10. These results demonstrate cell proliferation of the J774.A1
35 334 macrophages in contact with iron-based MOFs and further studies will be necessary to decipher
36 335 the involved mechanism. However these preliminary results showed that materials were not
37 336 cytotoxic.
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3. CONCLUSION

An efficient and reproducible method for the synthesis of copolymers of water-soluble $\text{PHO}_{(67)}\text{U}_{(7)}\text{SO}_3^-(20)\text{PEG}_{(6)}$ and $\text{PHO}_{(67)}\text{SO}_3^-(20)\text{PEG}_{(13)}$ has been developed by two successive photoactivated thiol-ene reactions. These two copolymers will be used for the coating of MOF-type nanoparticles. The graft copolymers based on unsaturated PHAs have been heterofunctionalized with on one hand the polar SO_3^- sulfonate groups to promote ionic interactions with nanoMOF and on the other hand with PEG moieties to improve hydrophilicity. The molar composition of the copolymer is strictly controlled thanks to the efficiency of the thiol-ene reaction. The presence of sulphur detected by the STEM technique coupled with EDX attests to the effective coating of the MOF surface by the functionalized copolymers. The presence of PEG aims on the one hand to increase the stability of the system in a physiological environment and on the other hand to escape the phagocytic cells of the immune system. Moreover, nano-hybrid MOF based on PHA copolymers do not cause a significant toxicity reaction against J774.A1 macrophages. With further studies, the hybrid nanoparticles MOF- $\text{PHO}_{(67)}\text{U}_{(7)}\text{SO}_3^-(20)\text{PEG}_{(6)}$ and MOF- $\text{PHO}_{(67)}\text{SO}_3^-(20)\text{PEG}_{(13)}$ with a diameter close to 300 nm that are stable for at least 6 h in a buffered physiological environment, would constitute a new generation of biocompatible therapeutic nanovectors as innovative drug delivery system.

AUTHOR INFORMATION

Acknowledgments

The Authors would like to thank Eric LEROY and Remy PIRES for their technical assistance with the SEM-STEM EDX, Annick JACQ for the access to the ClarioSTAR system and Laurent MICHELY for his technical experiment. We are grateful to the financial support of IFREMER and Region Bretagne, France.

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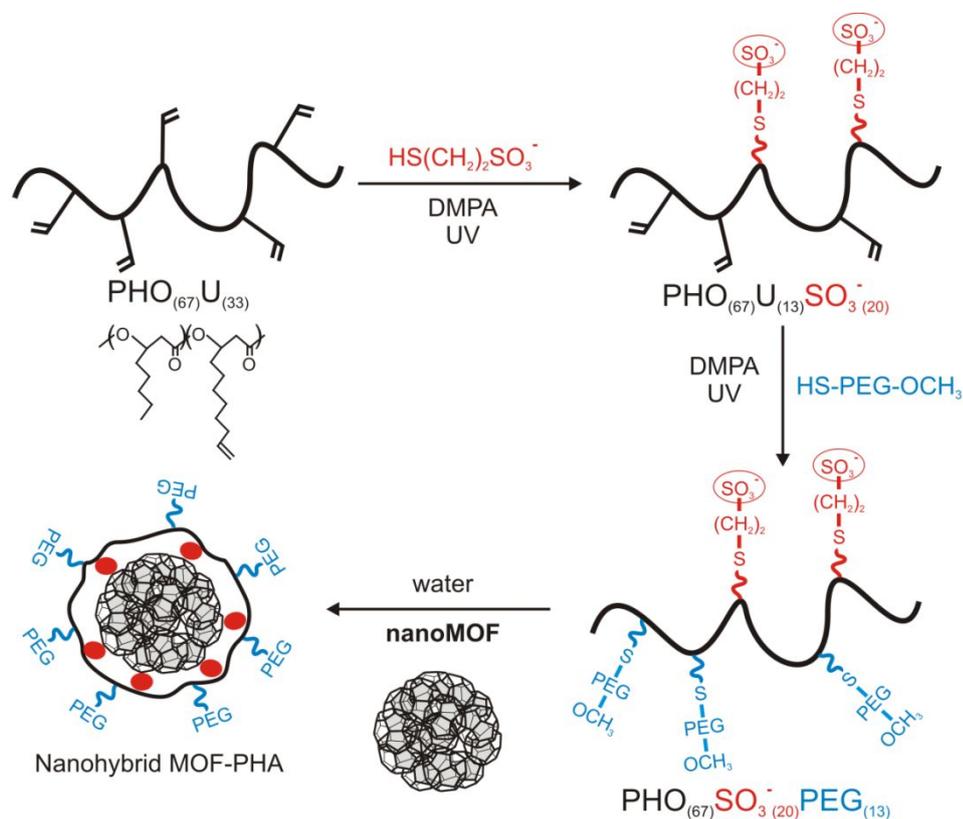
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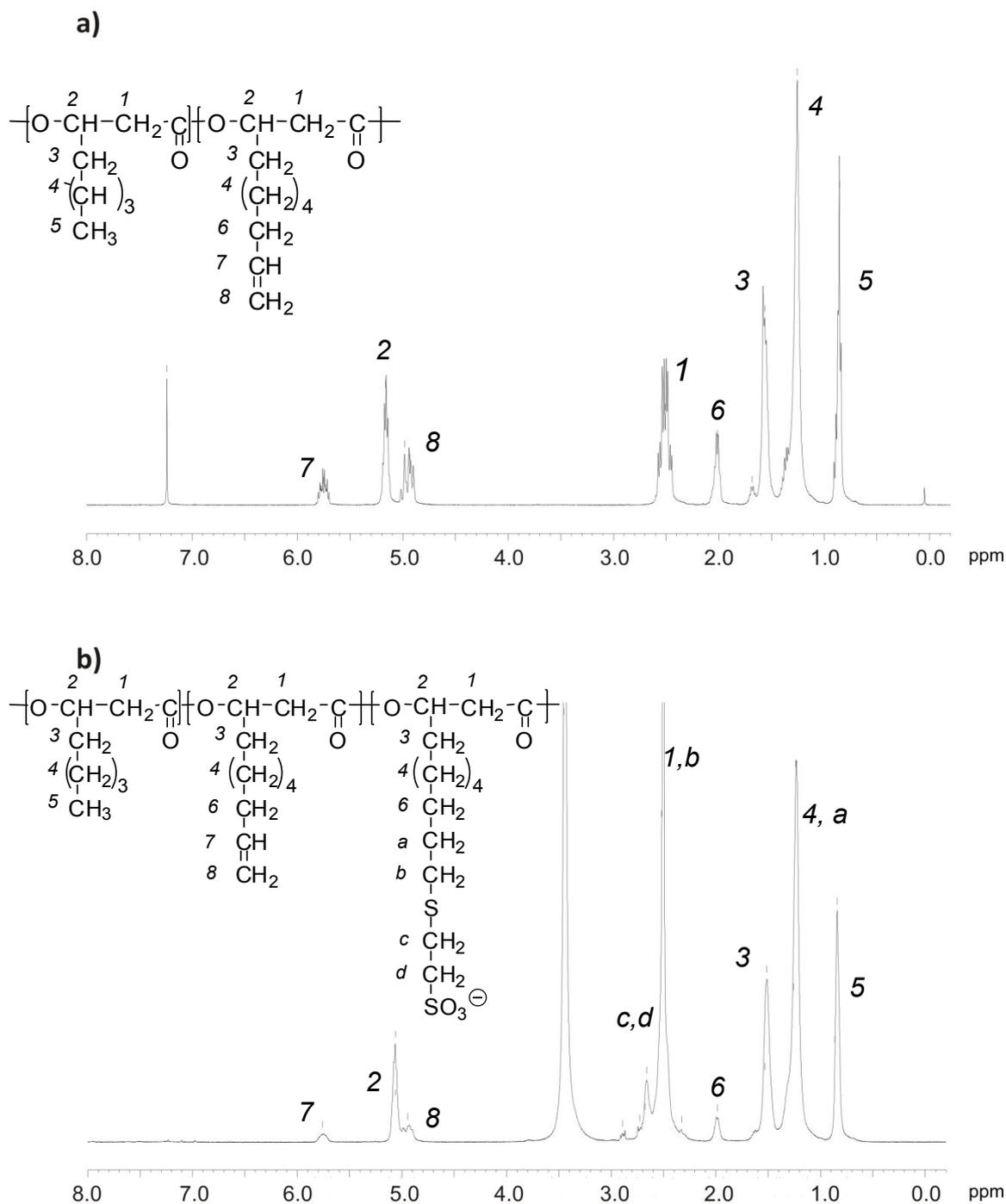
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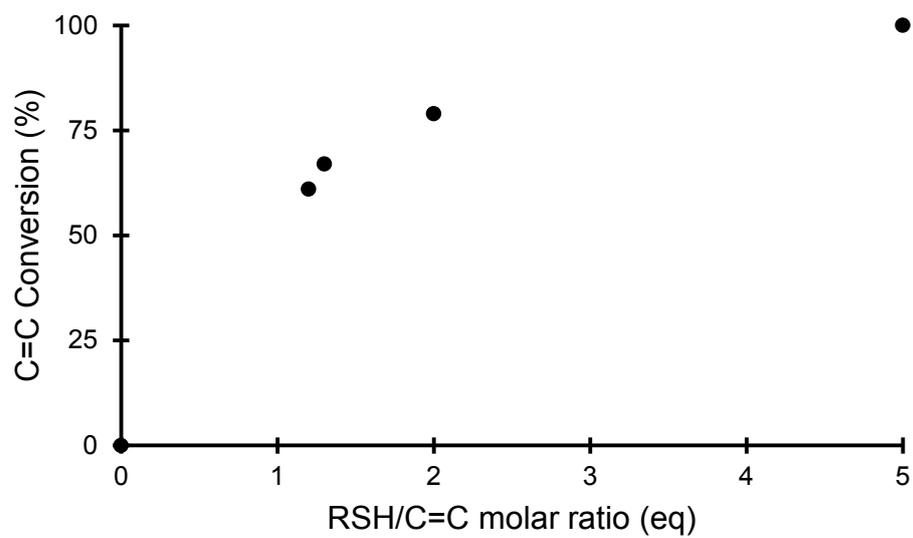
Figure 1. Elaboration of nanohybrid MOF-PHA



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542 **Figure 2.** ^1H NMR spectra. (a) $\text{PHO}_{(67)}\text{U}_{(33)}$ in CDCl_3 (b) $\text{PHO}_{(67)}\text{U}_{(13)}\text{SO}_3^-(20)$ in DMSO-d_6

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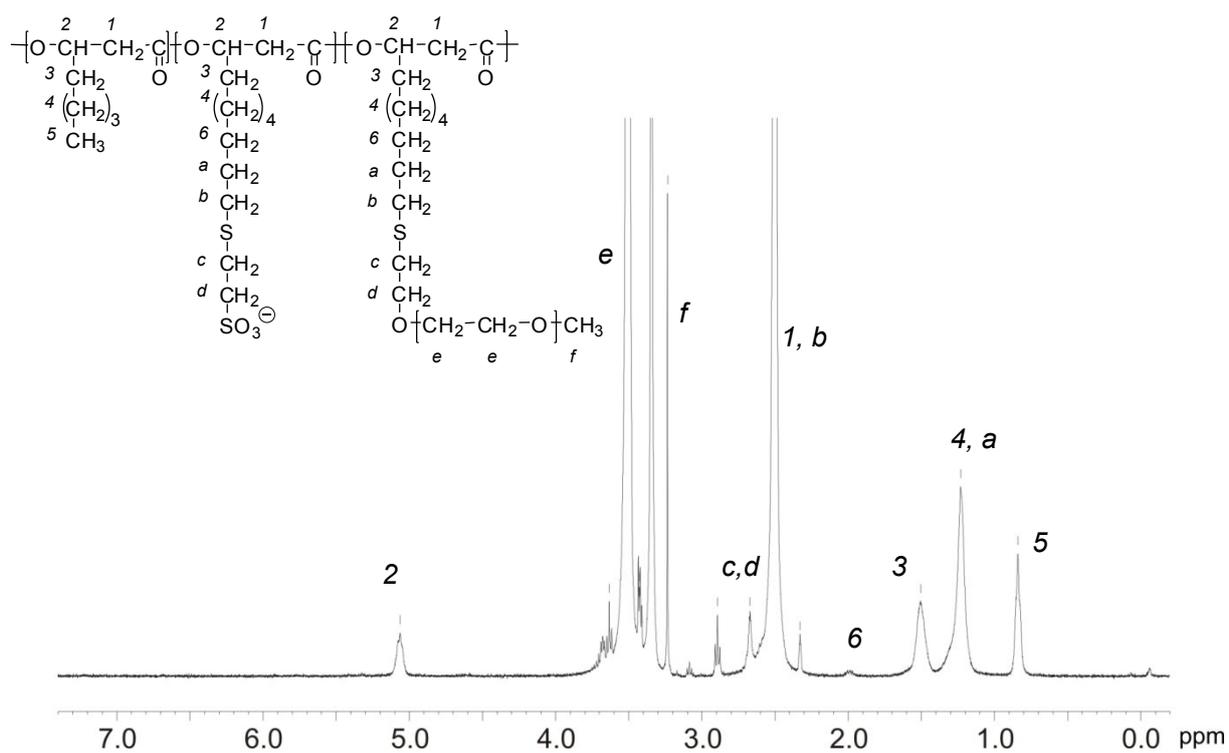


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Figure 3. C=C conversion of $\text{PHO}_{(67)}\text{U}_{(33)}$ determined by ^1H NMR

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Figure 4. ^1H NMR spectrum of $\text{PHO}_{(67)}\text{SO}_3^-(20)\text{PEG}_{(13)}$ in DMSO-d_6 .

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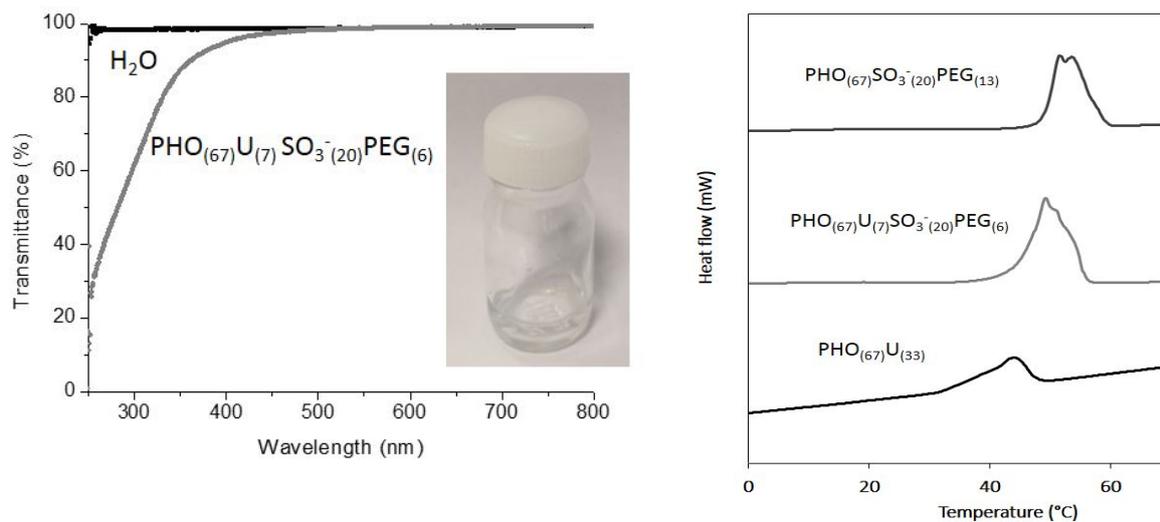
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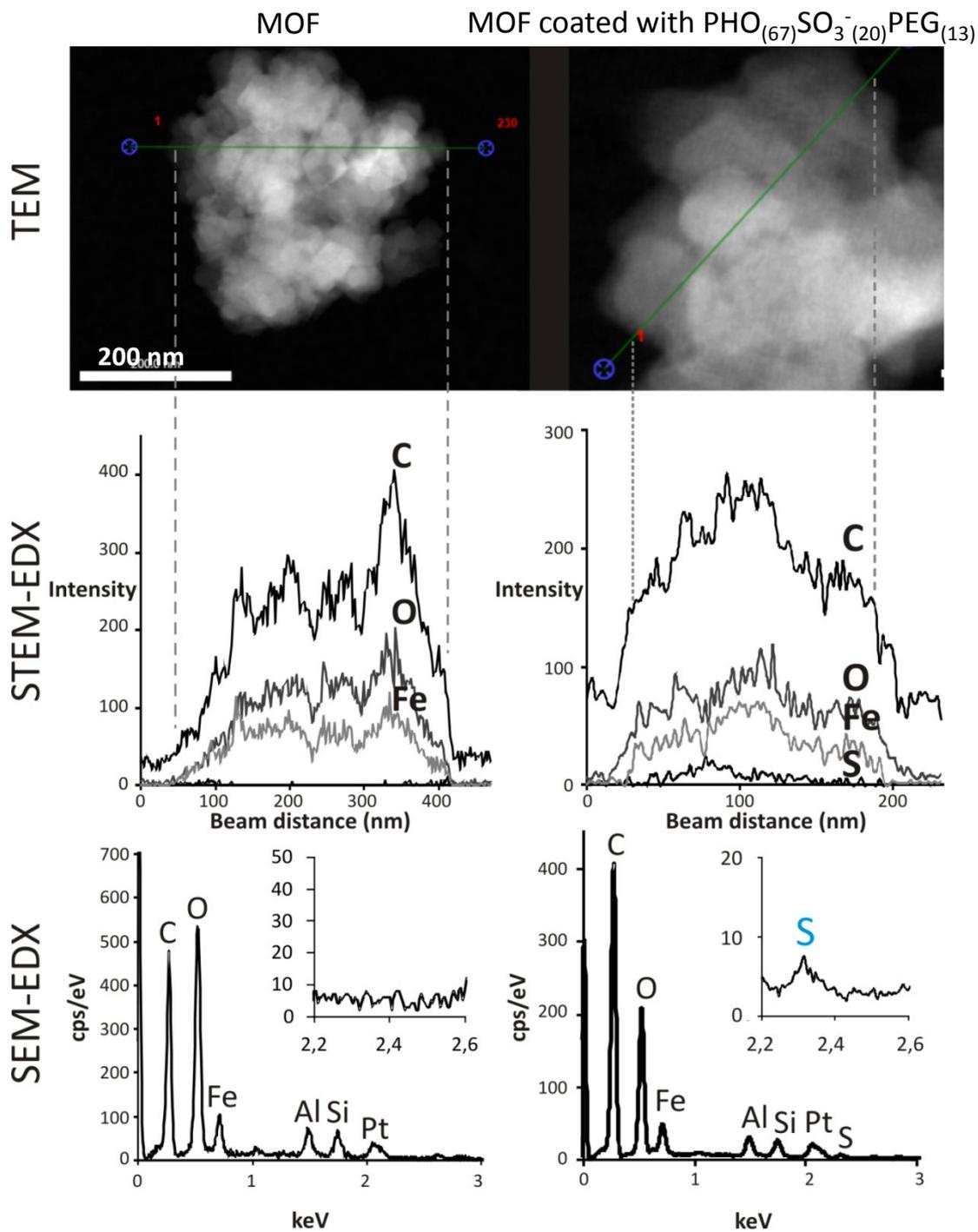


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557 **Figure 5.** Transmittance measurements in ultrapure water and DSC curves. Only the first
558 heating is reported.

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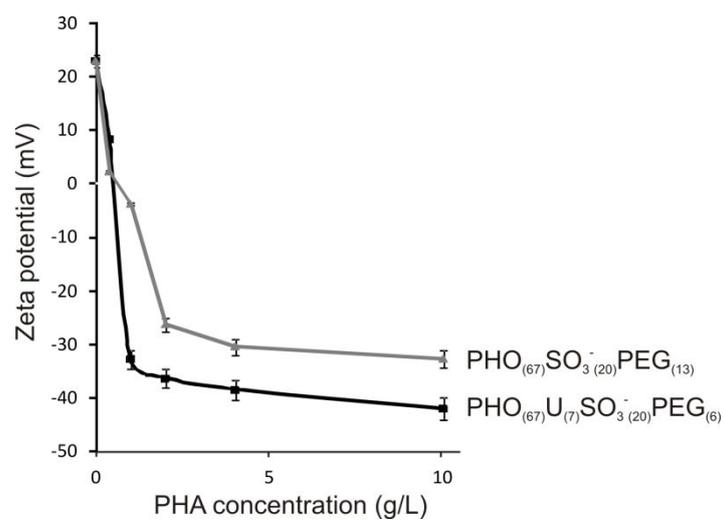
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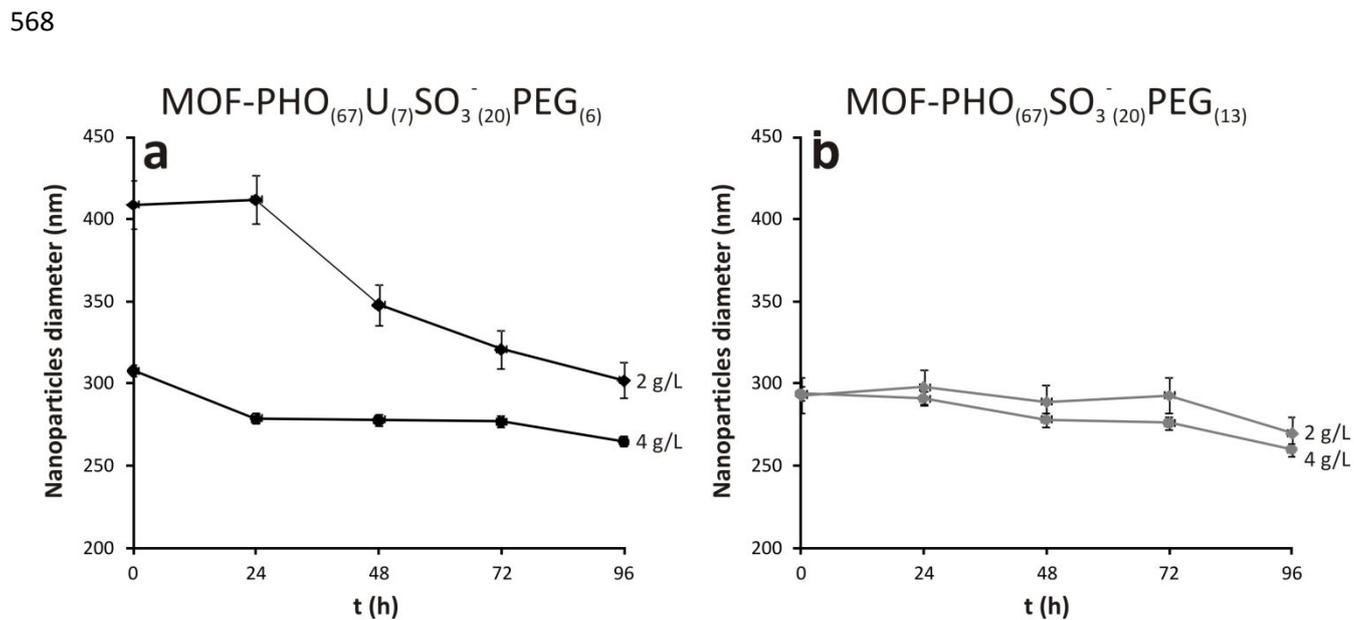
562 **Figure 6.** TEM, STEM-EDX and SEM-EDX analysis of free MOF nanoparticles and coated563 with $\text{PHO}_{(67)}\text{SO}_3^-(20)\text{PEG}_{(13)}$

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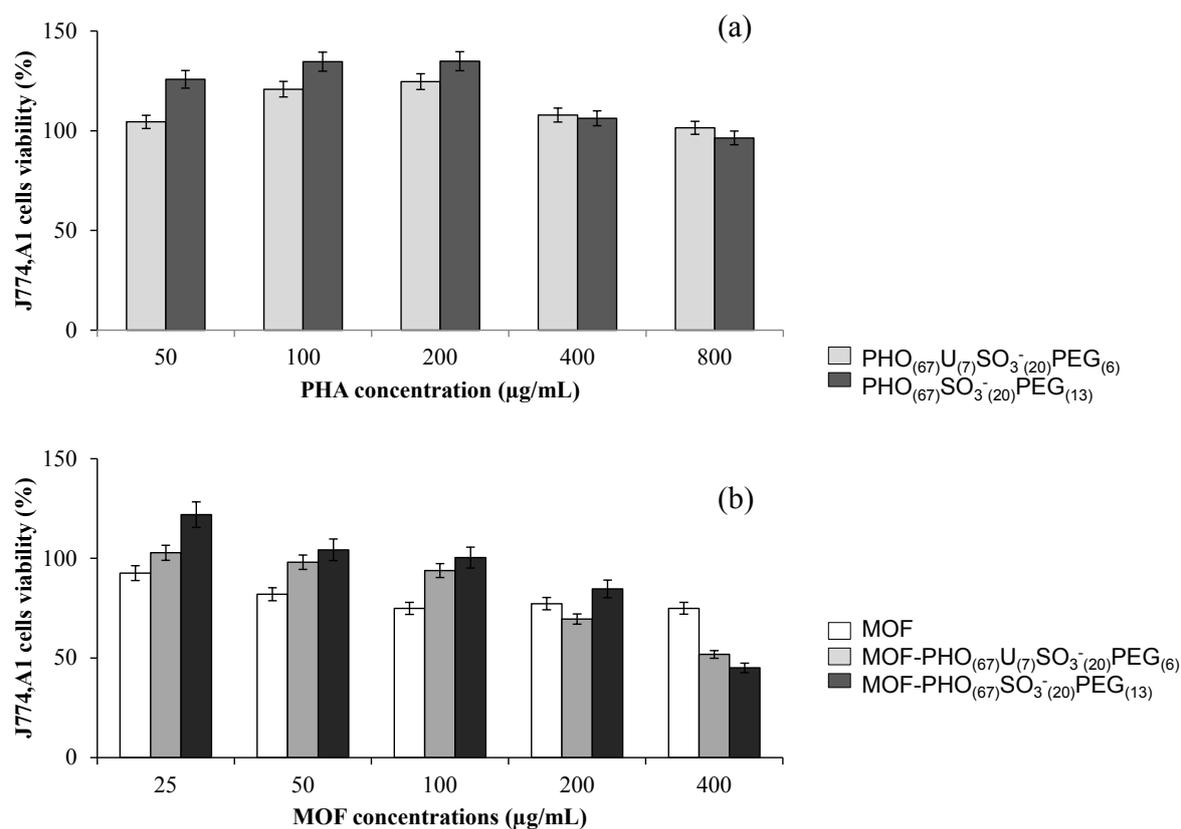


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566 **Figure 7.** Zeta potential of MOF nanoparticles coated with $\text{PHO}_{(67)}\text{SO}_3^-(20)\text{PEG}_{(13)}$ and
567 $\text{PHO}_{(67)}\text{U}_{(7)}\text{SO}_3^-(20)\text{PEG}_{(6)}$

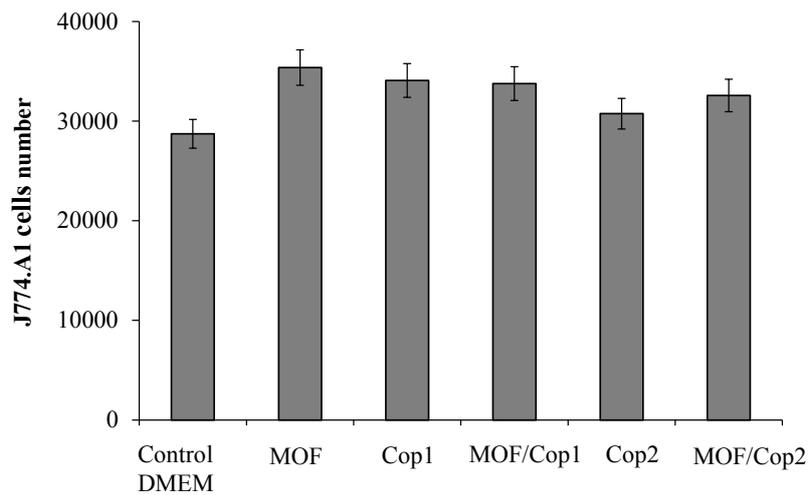


23 569 **Figure 8.** MOF-PHA hybrid nanoparticles diameters measured for 96 h in ultra pure water at
24 different copolymer concentrations.
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572 **Figure 9.** Viability of J774.A1 cells calculated using an LDH test, as a function of the
 573 concentrations of copolymers (a) or nanoMOFs, native or coated with functionalized PHA (b),
 574 after 24 hours of incubation. DMEM was used as control (100%). Experiments were done in
 575 triplicate.



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577 **Figure 10.** Number of viable J774.A1 cells determined by the RealTime Glo test for 42 h in
578 presence of MOF and coated MOF by $\text{PHO}_{(67)}\text{U}_{(7)}\text{SO}_3^-(20)\text{PEG}_{(6)}$ noted Cop1 or $\text{PHO}_{(67)}\text{SO}_3^-$
579 $(20)\text{PEG}_{(13)}$ noted Cop2

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Table 1. Characterization of PEG, PHO₍₆₇₎U₍₃₃₎, PHO₍₆₇₎U₍₇₎SO₃⁻₍₂₀₎PEG₍₆₎ and PHO₍₆₇₎SO₃⁻₍₂₀₎PEG₍₁₃₎ polymers.

Samples	Molar masses		Thermal properties			
	Mn (g/mol)	PDI	Tg ^c (°C)	Tm ^d (°C)	ΔHm ^d (J/g)	
					PHA	PEG
PEG ₂₀₀₀	1,700 ^b	1.3 ^b	-	53	-	185
PHO ₍₆₇₎ U ₍₃₃₎	40,000 ^a	1.7 ^a	-40	44	14	-
PHO ₍₆₇₎ U ₍₇₎ SO ₃ ⁻ ₍₂₀₎ PEG ₍₆₎	24,000 ^b	1.1 ^b	-	49	-	82
PHO ₍₆₇₎ SO ₃ ⁻ ₍₂₀₎ PEG ₍₁₃₎	29,500 ^b	1.1 ^b	-	52	-	132

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^a Determined by SEC in CHCl₃.

^b Determined by SEC in H₂O/LiNO₃

^c Recorded from the second heating of DSC.

^d Recorded from the first heating of DSC, for copolymers ΔHm was calculated using the formula $\Delta H = \Delta H_i / W_i$, where ΔH_i is the area of the DSC endothermic peak, and W_i is the weight of PHA or PEG determined by ¹H RMN.

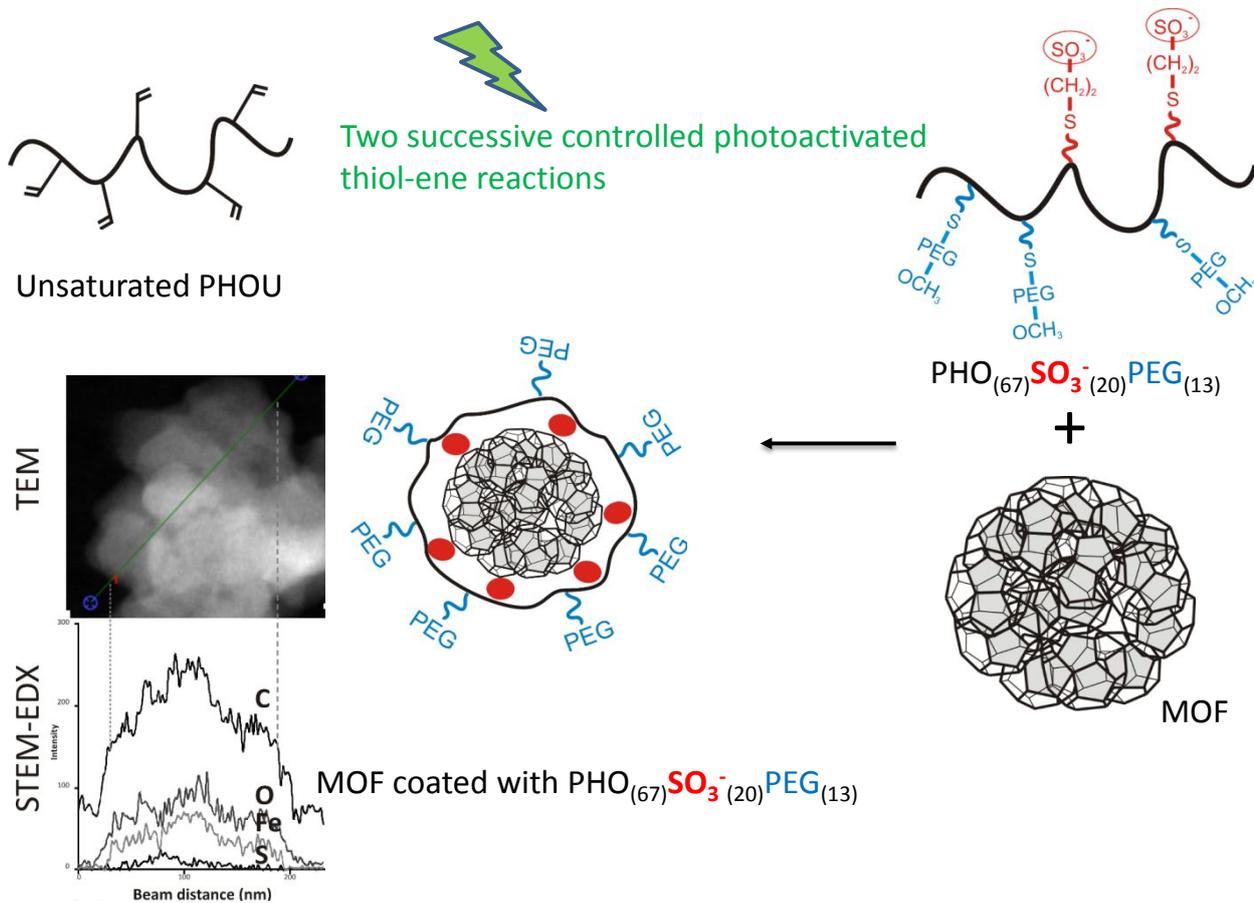
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Table 2. Average atomic composition of MOF nanoparticles determined by STEM or SEM-EDX

Nanoparticles		C (%)	O (%)	Fe (%)	S (%)	Ratio C/Fe	Ratio S/Fe
STEM-EDX	MOF	74.6	18.8	6.4	0.0	11.6	0.00
	MOF- PHO ₍₆₇₎ U ₍₇₎ SO ₃ ⁻ ₍₂₀₎ PEG ₍₆₎	76.7	16.6	5.9	0.8	12.9	0.13
	MOF- PHO ₍₆₇₎ SO ₃ ⁻ ₍₂₀₎ PEG ₍₁₃₎	77.7	16.4	5.2	0.8	15.1	0.16
SEM-EDX	MOF	55.2	38.3	6.5	0.0	8.5	0.00
	MOF- PHO ₍₆₇₎ U ₍₇₎ SO ₃ ⁻ ₍₂₀₎ PEG ₍₆₎	69.2	23.0	6.9	0.9	10.0	0.13
	MOF- PHO ₍₆₇₎ SO ₃ ⁻ ₍₂₀₎ PEG ₍₁₃₎	70.0	22.9	6.3	0.8	11.1	0.13

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