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Assessment of nanoplastic extraction from natural samples for quantification purposes

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Highlights

- Evaluation of nanoplastic retention capacities during membrane filtration
- Polystyrene nanospheres, selected as model nanoplastics, were used to monitor the losses
- Polystyrene nanospheres are strongly retained on polymer-based membranes or glass fiber filters
- Plane inox grids have very low retention capacities
- Monitoring of the nanometric fraction during river sample by nanotracking analysis

Abstract

Plastic pollution poses an increasing threat to the whole ecosystem. Scientists recently realized that this pollution also occurs at the nanoscale. Nanoplastic monitoring is a real challenge and published data are scarce. Above the use of appropriate analytical instrument, one main obstacle is to quantitatively isolate the nanoplastic from larger particles in real samples. We propose a method based on the monitoring of polystyrene

29 nanospheres following the polystyrene intrinsic fluorescence to assess the losses.
30 Polymer-based membranes were tested for filtration, ultrafiltration and dialysis;
31 important losses were observed. The isolation of the colloidal fraction is proposed
32 using stainless steel grid with reduction of the losses. The ability of glass fiber filters to
33 trap the nanoplastic was also evaluated as a mean to transfer the particle to the
34 analytical instrument. As an illustration, a river sample was processed with the
35 recommended protocol and the colloidal content monitored by nanoparticle tracking
36 analysis. As a conclusion, we insist on the fact that it is absolutely necessary to
37 evaluate nanoplastic extraction efficiencies for quantification purposes and we give
38 recommendations to limit losses.

39

40 **Capsule**

41 **In order to achieve nanoplastics quantification in environmental, a simple**
42 **protocol to assess nanoplastics losses during extraction is presented.**

43

44 **Introduction**

45

46 Plastic pollution is a growing topic of public concern. To address the questions of
47 society, research has intensified to better evaluate the effects of plastic on ecosystems
48 and human health. Analytical developments were proposed to characterize plastic
49 pollution at the micro- and nano-scales (Filella, 2015). The analysis of nanoplastic
50 (NP, particles below 1 μm) is still very challenging. Technological developments in
51 terms of characterization and detection were recently reviewed (Schwaferts et al.,
52 2019). Physicochemical characterization and investigation of NP behavior in the
53 environment are also better understood (Besseling et al., 2017; Gangadoo et al., 2020;
54 Pradel et al., 2020; Wu et al., 2019).

55 To study NP fate and behavior, many laboratory tests have been performed with model
56 particles (Gangadoo et al., 2020) and typically with polystyrene nanospheres (also
57 called polystyrene latex, PSL). PSLs are used as model particles because they are
58 commercially available in a wide range of sizes and with various functionalizations
59 (Chae and An, 2017). PSL is usually available as formulated aqueous dispersions and
60 the presence of surfactants leads to very stable dispersions, preventing aggregation

61 and sedimentation. To proceed with tests, PSLs are classically diluted in water; or
62 sometimes in DMSO (Jeong et al., 2018). After dilution, the dispersions are usually
63 vortexed, less frequently sonicated (Dong et al., 2018). Often, the dispersions are
64 used as they are in exposure media. To remove the preservatives, it was sometimes
65 proposed that the PSL dispersions be centrifuged (Cole and Galloway, 2015), filtered
66 (Greven et al., 2016) or dialyzed (Mattsson et al., 2015; Mattsson et al., 2017; Pikuda
67 et al., 2019). After these preparation steps, PSL concentrations were sometimes
68 controlled by nanoparticle tracking analysis (NTA) to provide concentrations
69 (expressed in number of particles/mL) (Mattsson et al., 2015; Mattsson et al., 2017)
70 or by fluorescence measurements (Pikuda et al., 2019) but the concentrations of the
71 particles were seldom monitored during these operations.

72 Even if polystyrene nanosphere purchased from market, might be far away from the
73 environmental samples, the use of PSL offers a fair number of advantages in
74 evaluation tests and will certainly still be heavily used in the future. However, very few
75 studies have investigated more environmentally relevant systems, such as
76 polydisperse and polymorphic mixtures of model particles (Baudrimont et al., 2020;
77 Pradel et al., 2020). The use of PSL allows us to draw an outline regarding NP fate
78 and behavior or interaction with organisms in the natural habitat while keeping in mind
79 that NP occurring in the environment are highly complex systems (Galloway et al.,
80 2017; Garvey et al., 2020; Gigault et al., 2018; Roweczyk et al., 2020; ter Halle and
81 Ghiglione, 2021).

82 There are only very few studies reporting NP analysis in natural samples (Davranche
83 et al., 2020; Mintenig et al., 2018; Sullivan et al., 2020; ter Halle et al., 2017; Wahl et
84 al., 2021; Zhou et al., 2019). The sampling strategies proposed to extract and
85 concentrate NP were never properly evaluated and the possible losses overlooked.
86 Ter Halle et al. proposed the pre-filtration of seawater on 5 μm polyethersulfone (PES)
87 membranes to isolate the colloidal fraction, which was then concentrated by frontal
88 ultrafiltration (20 kDa PES membranes) for DLS characterization (ter Halle et al.,
89 2017). The dispersion then underwent lyophilization. NP were trapped in the remaining
90 sea salts and directly analyzed by pyrolysis-gas chromatography-mass spectrometry
91 (Py-GC-MS). This approach was only applied to detection, and the possible losses
92 during this multiple step sample preparation were not evaluated. The evaluation of NP
93 recovery during sample preparation was only reported once upon ultrafiltration on PES

94 (Mintenig et al., 2018); recoveries were limited to 54% with PES membrane. Sullivan
95 et al. recently analyzed NP in river water (Sullivan et al., 2020). Without pre-filtration,
96 they directly proceeded to frontal filtration of 200 mL of river water on a
97 polytetrafluoroethylene (PTFE) membrane (0.45 μm porosity). The particles trapped
98 on the filters were introduced, after cryomilling, into the pyrolysis chamber for
99 semiquantitative analysis by pyrolysis-gas chromatography time of flight mass
100 spectrometry (Py-GC-TOF). But the absence of prefiltration did not enable to
101 established the size range of the analyzed particles. PTFE membranes were often
102 used in pre-filtration step to isolate the NP from microplastics (Davranche et al., 2020;
103 Materić et al., 2020; Wahl et al., 2021) but the retention capacity of the PTFE
104 membrane was never estimated. As the number of studies quantifying nanoplastic in
105 natural sample is increasing (Cai et al., 2021), future work must include the evaluation
106 of NP recoveries during sample preparation.

107 The present study aims at evaluating PSLs (from 50 to 1000 nm) behavior upon various
108 preparation protocols, such as filtration, ultrafiltration or dialysis. The method to monitor
109 PSL was based on convenient, nondestructive and easy-to-handle fluorescence
110 measurements. As a pre-filtration step, nylon single strand tissue and stainless-steel
111 were compared to polymer-based membranes. In order to trap and collect NP, we
112 evaluated glass fiber filters. Finally, a two-step protocol was proposed and tested with
113 a river sample and the colloidal content was monitored by NTA.

114 **Materials and methods**

115 **Materials and chemicals.** Polybead® polystyrene nanospheres (1000, 750, 500, 350,
116 200, 100 and 50 nm) were purchased as aqueous solutions at 2.6% w/v (Polysciences,
117 Warrington, USA). The membranes used were 47 mm diameter and of different
118 polymer-based natures: polycarbonate, PC (Fischer Scientific, Illkirch-Graffenstaden,
119 France), PES (Sterlitech, USA), cellulose acetate, CA (Sterlitech, USA), nylon single
120 strand (Sefar Nitex®, VWR, Fontenay-sous-Bois, France), hydrophilic PTFE
121 membranes (Omnipore, Merck Millipore, Molsheim, France), stainless-steel grid “plain
122 dutch weave” (Negofiltre, Moret-Loing-et-Orvanne, France) and glass fiber filters (GF/F
123 Whatman®, Merck Millipore, Molsheim, France). Ultra-pure water (18.2 m Ω .cm, Milli-
124 Q filtration unit, Merck Millipore, USA) was used for solution preparation. Glass filtration
125 units were purchased from Verre Wagner (Toulouse, France). Frontal ultrafiltration
126 measurements were carried out in Amicon® 400 mL stirred cells (Aldrich, Saint

127 Quentin Fallavier, France). Tangential ultrafiltration was carried out on Akta® flux S
128 (GE Healthcare, Vélizy-Villacoublay, France). For frontal ultrafiltration, the membrane
129 used was PES type BU50 at 50 kDa (Alting, Hoerd, France). Tangential ultrafiltration
130 was carried out on autoclavable PES at 50 kDa, hollow fiber cartridge UFP-50-C-
131 MM01A (GE Healthcare, Vélizy-Villacoublay, France). Dialysis cells were from the
132 brand Tube-O-Dialyze mini dialysis system with membrane presenting a cut off at 1
133 kDa (Merck Molsheim, France).

134 **Filter calcination.** The glass fiber filters were prepared by calcination to remove any
135 polymer or organic compound traces with a heating ramp from room temperature to
136 500 °C at a rate of 80 °C/hour with a hold of 30 hours at 500 °C in a furnace
137 (Nabertherm® LV052K1RN1). Stainless steel grids were calcined at 500°C for 3 h with
138 a heating ramp of 200°C/h from room temperature in the same furnace.

139 **PSL dispersion preparation.** The unfunctionalized PSL were named PS, and the
140 carboxyl- and amino-terminated PSL particles were named PSCOOH and PSNH₂,
141 respectively. The size was indicated afterward (PS-50 for 50 nm unfunctionalized
142 beads). PSL dispersions were obtained after diluting the commercial formulations to
143 reach 10 ppm by three successive dilution steps (dilution factor of 2600). Before any
144 characterization or manipulation (filtration, dialysis, ultrafiltration), the diluted
145 dispersion was sonicated for 30 minutes at 25°C. We ensured that sonication, in our
146 experimental setup, did not alter the fluorescence emission of the beads and that the
147 bead sizes were not altered by diffusion light scattering (DLS) measurements for
148 PSCOOH-500, PSCOOH-350 and PSCOOH-50. The nominal size stated by the
149 manufacturer were confirmed by DLS measurements. The z-averages were measured
150 at 50.3 ± 1.1 nm, 97.7 ± 0.6 nm, 361.3 ± 5.5 nm and 520.5 ± 25.7 nm for PSCOOH-50
151 PSCOOH-100, PSCOOH-350, and PSCOOH-500, respectively. Tests were
152 systematically run with freshly diluted dispersions. When we run the stability
153 evaluation, they were stored at 4°C.

154 **Gravimetric filtration.** The filtrations were carried out under vacuum with a glass unit
155 equipped with 0 porosity sintered glass (corresponding to pores between 160 and 250
156 µm) with a diameter of 47 mm to hold the membranes. For the filtration, systematically
157 a volume of 10 mL was used and filtered in 3 minutes. Membranes were tested with
158 nanospheres bigger or smaller than their reported pore sizes. Nanospheres
159 concentrations were measured using the calibrations curves presented in figure SI2

160 and SI3 before and after each operation. The retention capacity was calculated by the
161 difference of concentration measured before and after filtration. Tests performed with
162 6 replicates presented retention capacities with a RSD of 10%.

163 **Dyalysis.** The cells used had a volume of 5 mL with 1 kDa cut off membrane (Merck
164 Molsheim, France). A volume of 3 ml of nanosphere dispersion was placed in the
165 dialysis cell which was placed in a 5 L beaker for 48 h. The retentate was analyzed by
166 fluorescence in order to estimate how much nanospheres were recovered after the
167 operation.

168 **Ultrafiltration.** Frontal ultrafiltration was carried out with 100 mL of dispersion at 1 bar.
169 Tangential ultrafiltration was carried out with 100 mL of dispersion at 50 mL.min⁻¹, with
170 transmembrane pressure at 0.5 bar and delta pressure at 0.22 bar

171 **Dynamic light scattering (DLS).** DLS was carried out at 25°C on a Malvern (Orsay,
172 France) Zetasizer NanoZS. Solutions were analyzed in triplicate without being filtered
173 to characterize the plain samples. Data were analyzed using the general-purpose
174 nonnegative least squares (NNLS) method. DLS measurements were carried out at
175 1000 ppm for the carboxyl functionalized particles with sizes from 50 to 1000 nm.

176 **Scanning electron microscopy (SEM).** After filtration, the membrane was dried at
177 room temperature for 48 hours. A piece of the membrane was cut and fixed on a
178 microscope stub with double-sided tape, then sputtered with platinum. Membranes
179 were examined on an FEI Quanta 250 FEG scanning electron microscope at an
180 accelerating voltage of 5-10 kV.

181 **Fluorescence.** Fluorescence measurements were performed at 25°C using a HORIBA
182 Jobin Yvon Fluorolog 2 IHR equipped with a temperature-controlled cuvette holder,
183 with an excitation wavelength of 250 nm and an emission wavelength of 310 nm. The
184 6 points calibration curves were obtained from the 10 ppm solution after proper dilution.
185 Calibration points were recorded with fresh solutions and systematically recorded the
186 same day as the filtration tests. In order to ensure that the signal was systematically
187 properly integrated, we monitored PSL concentrations above a limit of quantification
188 set at 1 ppm regardless of the nanosphere size or functionalization.

189 **Sampling of river water.** The water sample from Garonne River was collected on 16
190 September 2021 directly from the bank of the River (gps coordinates: 43°34'38.1"N

191 1°26'08.8"E) in a 2 L glass bottle. The water temperature was around 22°C, and the
192 turbidity around 21 NTU. The river sample was processed with the two steps filtration
193 on 5 µm stainless steel grid and glass fiber filter directly after sampling without storage
194 delaying.

195 **Nanoparticle tracking analysis (NTA).** Colloidal contents and size distribution of river
196 water samples were assessed by NTA using a Malvern NanoSight NS300® (Malvern
197 Instruments Ltd, UK) at 25°C. The experiments were performed in triplicate under a flow
198 of 0.1 mL.min⁻¹ during 90 sec. The conditions were checked to have an optimal particle
199 concentration in the microscope field of view between 10⁷ and 10⁹ particles.mL⁻¹. The
200 presented result consisted in the merging of the triplicats.

201 **Results and discussion**

202 **Fluorescence measurements**

203 Standard PSL presents an intrinsic fluorescence upon UVC excitation. Although this
204 fluorescence is far less intense compared to biolabeled beads, the difference in cost
205 makes this method useful and convenient. After dilution at 10 pmm, when repeatedly
206 analyzing a given PSL dispersion, the fluorescence emission presented a relative
207 standard deviation (RSD) of 2 % (n=8 with PSCOOH-50). The repeated dilution from
208 a commercial formulation was also reproducible; the RSD were below 8% (n=6) with
209 the PSL tested (PSCOOH-50, PSCOOH-100, PSCOOH-500, PSNH₂-500 and PS-
210 500).

211 Using polystyrene intrinsic fluorescence emission as a basis for quantification, we
212 evaluated if there was a linear relationship with PSL concentration. The calibration
213 curves presented excellent linearity with correlation factors greater than 0.97 with all
214 tested PSL (Figure SI2 and Figure SI3). The reproducibility of the calibration curves
215 was very good when repeating the standards preparation (use of PSCOOH-100, n=5,
216 no significant differences with Student's t-test at 5%).

217 The PSL intensities of fluorescence were size-dependent; the bigger beads being more
218 fluorescent. This interesting property was recently observed by Konemann et al. but
219 was very difficult to rationalize (Konemann et al., 2018). One suggestion would be that
220 there was more fluorescence loss with smaller particles due to increasing light
221 scattering. It is important to underline that we have designed this set of experiments

222 with a conservative mass with an initial concentration set at 10 ppm, whereas due to
223 the particle size differences, the number of particles were different from one test to
224 another when particle size varied.

225 Finally, when comparing nanospheres of the same size with different functionalization,
226 we observed differences. With the nanospheres at 100 nm, the intensity of
227 fluorescence decreased as: PSNH₂>PSCOOH>PS (Figure SI3). The differences were
228 less marked with 500 nm beads (Figure SI4). The functionalization of PS leading to
229 variations in the benzyl moiety spatial organization certainly impact the fluorescence
230 properties.

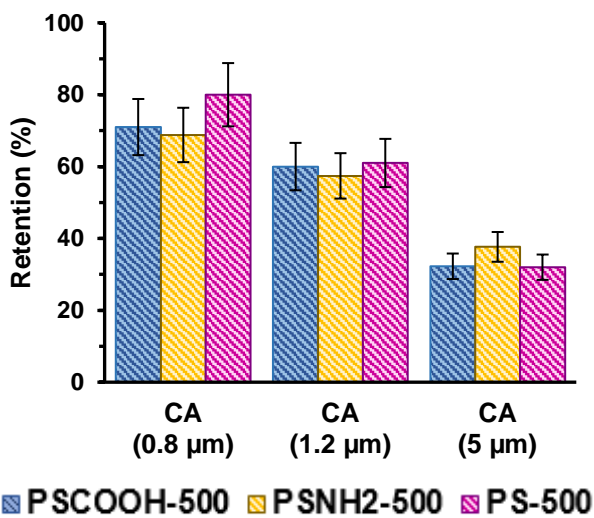
231 The supplier indicates a 6 months stability of the commercial dispersion. But there was
232 no indication about the stability after aqueous dilution. We evidenced that the diluted
233 dispersions at 10 ppm were not stable. After 7 days of storage at +4°C, PS-500
234 evidenced a 50 % decrease of the fluorescence (RSD=2.5%, n=3). The signal was
235 even weaker after 15 days. The DLS measurements did not indicate any changes in
236 the PS-500 hydrodynamic diameter. The same decay was measured after dilution with
237 PSCOOH-500 and PS-100. PS-50 appeared more stable with variations below 10%
238 over 7 days. After this first evaluation, our first recommendation is to systematically
239 use freshly diluted dispersion and avoid storage.

240 **Membrane filtration**

241 The purpose of this series of test was to provide a protocol to isolate the colloidal
242 fraction in a natural sample for quantification; in other word a procedure that would
243 allow to retain particles larger than 1 µm but quantitatively recover the smaller ones in
244 the filtrate. Membranes present distinct physicochemical characteristics implying
245 electrostatic interactions with particles together with variations in pore size uniformity
246 due to membrane construction and tortuosity. For all these reasons, retention cutoffs
247 are not absolute (Hernandez et al., 1996) and the experimental tests presented here
248 appeared necessary. The retention capacities were compared with strictly the same
249 operating conditions in terms of volume filtered and flow rate at the same mass
250 concentrations (10 ppm). This implies that, if comparing the conditions between the 50
251 and 500 nm diameter beads for example, the 50 nm beads were 1000 times more
252 numerous.

253 PS-500, PSCOOH-500 and PSNH₂-500 were retained in the same proportions for a
254 given membranes. In total, CA, PVDF, PES, PC and PTFE membranes were tested at
255 pH 6 (Figure 1 to 3, difference in retention capacities were below 10%). The same
256 results were obtained with PS-100, PSCOOH-100 and PSNH₂-100 (data not shown).
257 We concluded that, under the present experimental conditions, transport through the
258 membranes was not driven by the functionalities of the beads, in other terms by
259 physicochemical interactions.

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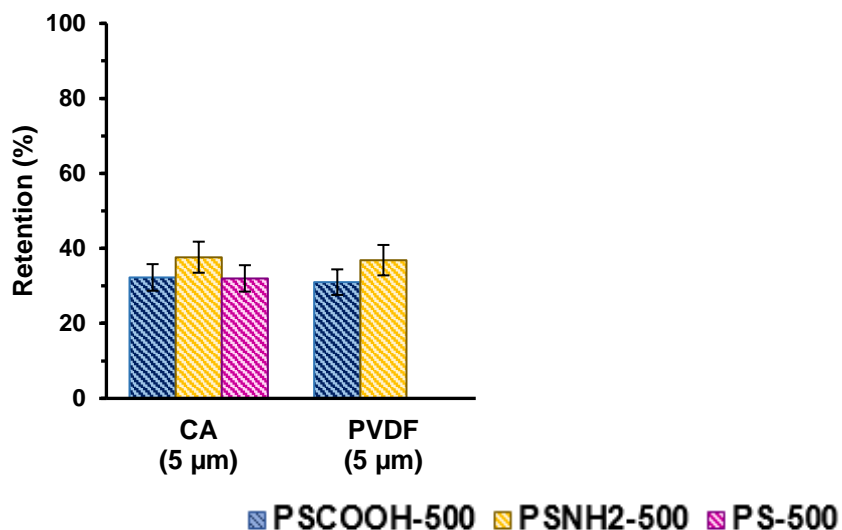
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266 Figure 1: Retention of 500 nm polystyrene nanospheres after filtration on cellulose
267 acetate membranes with various porosities. Polystyrene nanospheres were
268 functionalized with carboxylic acid or amine or were not functionalized. RSD were given
269 with n=6.

270 When 500 nm nanospheres were tested with membranes presenting greater pore
271 sizes (1.6 to 5 times), there was anyway membrane retention (Figure 1). For example,
272 membranes in CA or PVDF with a reported pore size of 5 μm retained 20 to 40% of
273 the 500 nm nanospheres (Figure 2). With a reported pore size of 1 μm, the PTFE
274 membrane retained approximately 90% of the 500 nm nanospheres (Figure 3 A). It
275 appeared that PTFE and PES presented higher retention rates than PC membranes
276 (Figure 3).



277

278

279

280 Figure 2: Retention of 500 nm polystyrene nanospheres after filtration on membranes
 281 with 5 μm porosities either in cellulose acetate (CA) or polyvinylidene difluoride
 282 (PVDF). RSD were given with n=6.

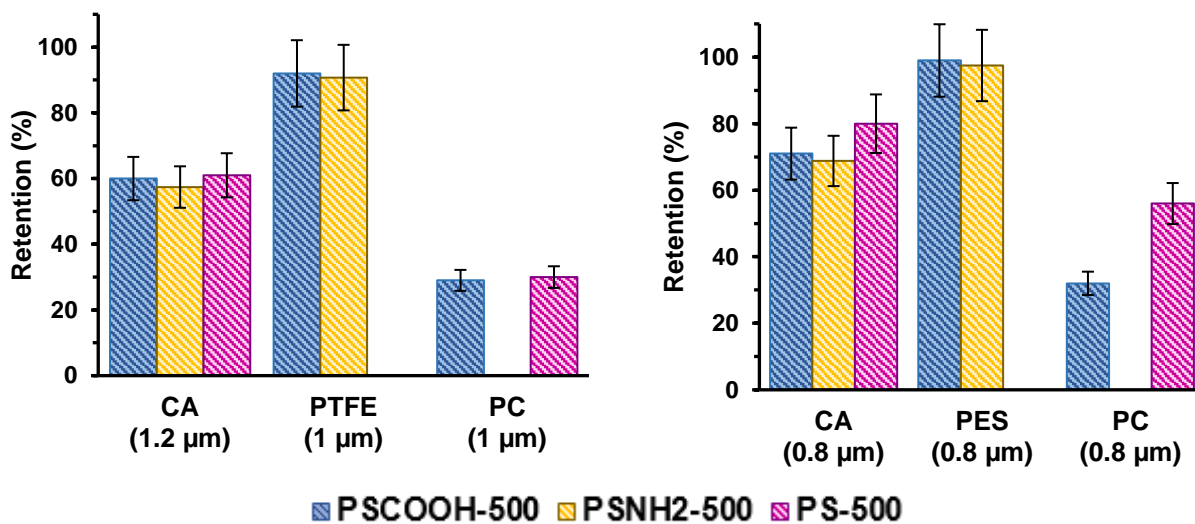
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A: 1 μm membranes

B: 0.8 μm membranes



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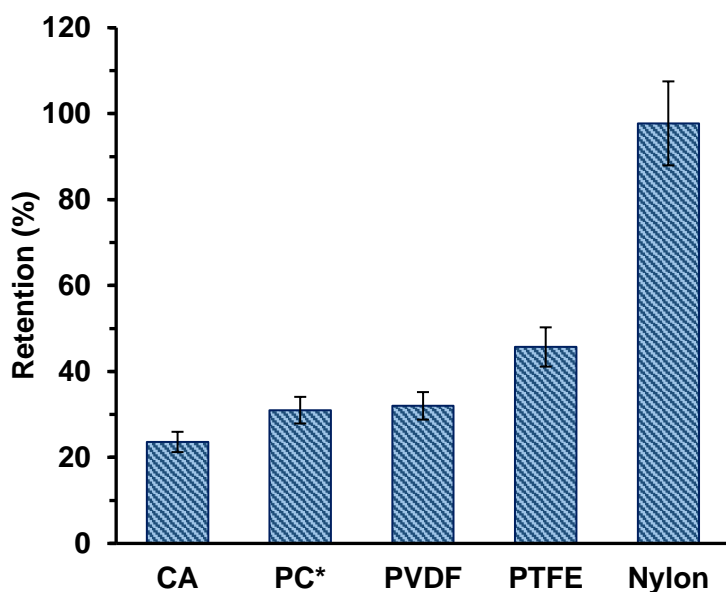
289 Figure 3: Retention of 500 nm polystyrene nanospheres after filtration on membranes
 290 with either 1 or 0.8 μm porosities. Membranes of cellulose acetate (CA), polyvinylidene
 291 difluoride (PVDF), polyethersulfone (PES) and polytetrafluoroethylene (PTFE) were
 292 tested. RSD were given with n=6.

293

294 Testing 350 nm nanosphere with larger pore size membranes, we also observed
295 important retention capacities. For example, 0.45 μm membranes in cellulose acetate
296 retained 94% of the PSCOOH-350 (Figure SI5). Testing even larger membrane pore
297 sizes, over 1 μm , the retention capacities for the PSCOOH-350 were above 20 to 25
298 %, all membrane type tested (Figure SI5).

299 PSCOOH-100 was tested with various membranes type with pore sizes at 0.45 and
300 0.4 μm (Figure 4). Again, there were important differences depending on the polymer-
301 based membrane. The 0.45 μm nylon membranes presented almost quantitative
302 retention. The 0.45 μm PTFE membrane was the second membrane, retaining a
303 substantial percentage of the particles (45%). On CA, with 0.7 to 0.8 μm pore size,
304 between 15 to 32% of PSCOOH-100 was retained (Figure SI6). With the use of even
305 larger pore size membranes (1 to 1.2 μm), the retention capacities were between 12
306 % (CA, Figure 2) and 30% (PC, Figure SI6). The retention capacities of the membranes
307 appeared to be ruled both by the pore size but also by the nature of the polymer used.

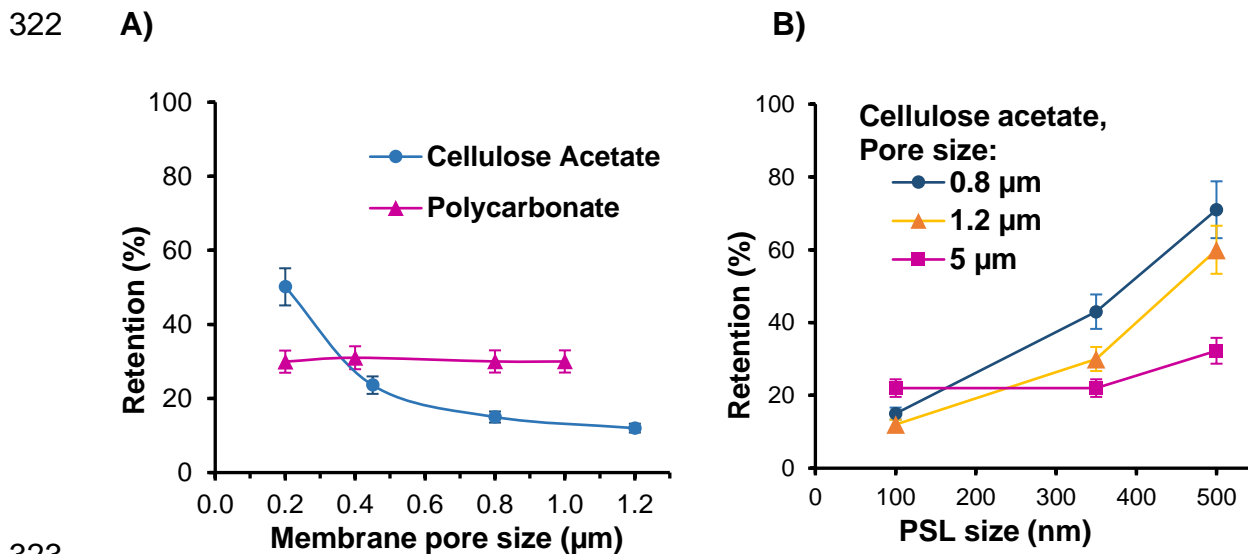
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310 Figure 4 : Retention of 100 nm carboxylated polystyrene nanospheres after filtration
311 on 0.45 μm membranes. Membranes of cellulose acetate (CA), polycarbonate (PC),
312 polyvinylidene difluoride (PVDF), polytetrafluoroethylene (PTFE) and nylon were
313 tested. The membrane marked with a star had a 0.4 μm porosity. RSD were given with
314 n=3.

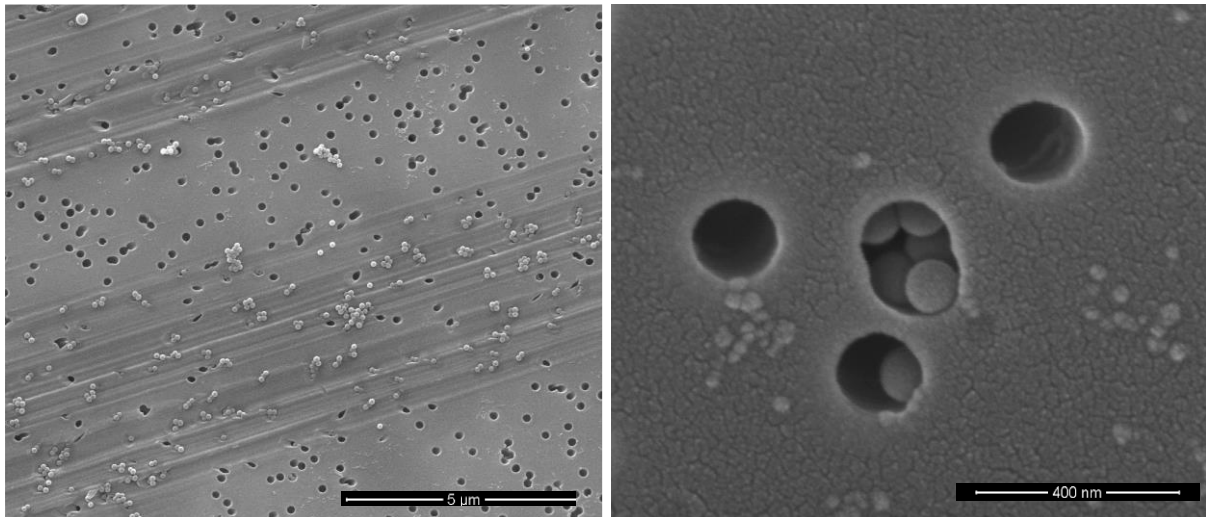
315 We thus considered a given membrane type with varying pore sizes using PSCOOH-
316 100 (Figure 5 A). With CA, the smaller the porosity, the higher the proportion of
317 particles trapped, ranging from 50% retention with 0.2 μm to 12% with 1.2 μm porosity.
318 With PC, the behavior was radically different because the percentage of retention was
319 30%, regardless of the pore size (from 0.2 to 1 μm). This difference was difficult to
320 rationalize but microscopic imaging presented below helped to apprehend the very
321 distinct structure of the polymer-based membranes tested.



323 Figure 5: A) Retention of 100 nm PS nanospheres after filtration on cellulose acetate
324 or polycarbonate membranes with increasing porosity. B) Retention of carboxylated
325 polystyrene nanospheres after filtration on cellulose acetate membranes with various
326 nanosphere sizes and membrane porosities. RSD were given with $n=3$.

328 Scanning electron micrographs (SEM) provided interesting information. This technic is
329 nonetheless relevant when polystyrene nanospheres are used and certainly more
330 difficult to interpret with real samples. SEM was informative about the morphologies of
331 the membranes and how the beads could be retained on them. PC membranes with a
332 porosity of 0.2 μm appear to have a relatively planar surface with holes approximately
333 0.2 μm wide. It appears that the 100 nm beads can be retained on the membrane
334 surface (Figure 6). Enlargement (Figure 6, on the right) shows that several beads can
335 enter the same pore and be retained inside it, thereby clogging the pore. This
336 configuration could explain why the 0.2 μm PC membrane retained some PS-100; the
337 retention capacities were of 30%. The CA membranes with the same porosity (0.2 μm)
338 had a very distinct organization, with multiple layers (Figure 7A). The multiple layer

339 morphology could explain why the CA membranes retained a larger fraction of the
340 beads with retention of 50%. The CA membrane with a porosity of $0.45\ \mu\text{m}$ had a very
341 different organization from the $0.2\ \mu\text{m}$ CA (Figure S17). We recommend if selecting a
342 given membrane for an extraction procedure development to visualize the membrane
343 structure because it is very informative.



344

345

346

347 Figure 6: Scanning electron micrographs of a $0.2\ \mu\text{m}$ polycarbonate membrane after
348 filtration of 100 nm carboxylate polystyrene nanospheres. The image on the right (B)
349 is an enlargement over a few pores, where several 100 nm beads can be observed as
350 trapped in a single pore (approximately 200 nm wide).

351 The PTFE membranes presented another organization (Figure 7B) and were made of
352 entangled fibers of distinct diameters. In Figure 7B, numerous PSCOOH-100
353 molecules were visible and seemed trapped in the fibrous morphology. Indeed, PTFE
354 membranes presented high retention capacities compared to the other membranes
355 tested.

356

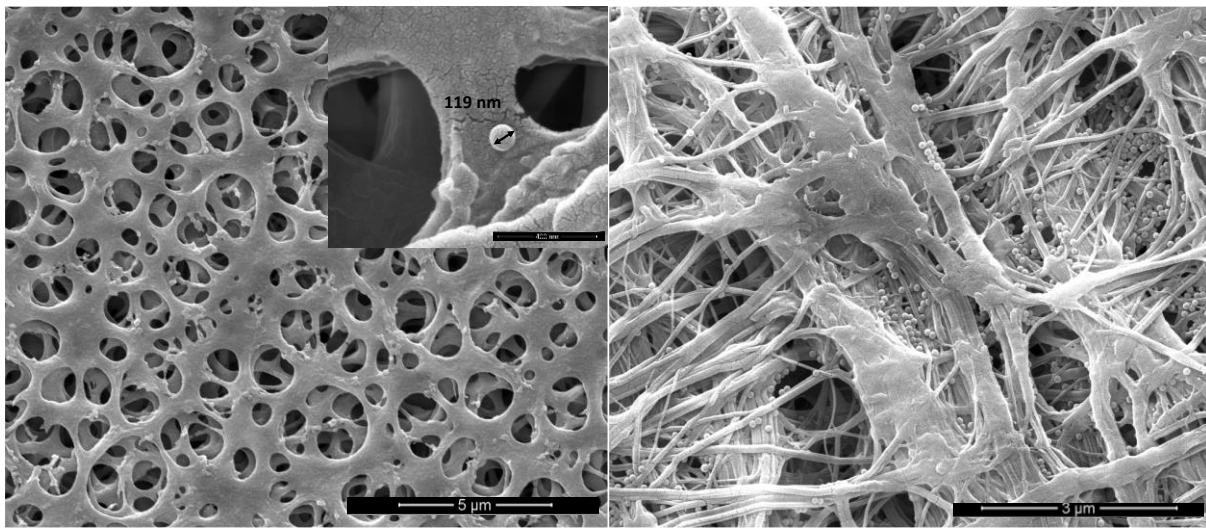
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360 A

B



361

362 Figure 7: Scanning electron micrographs of A) 0.2 µm cellulose acetate membrane and
363 B) 0.45 µm polytetrafluoroethylene after filtration of 100 nm carboxylated polystyrene
364 nanospheres.

365

366 Finally, PSCOOH-50, the smallest PSL tested, were filtered through 0.2 µm
367 membranes (Figure SI8). The percentages of retention were between 6 and 14%, and
368 the differences between membrane types were not significantly different.

369 Altogether, it appeared that the filtration process using polymer-based membranes was
370 crucial in terms of PSL retention. We observed very important retention capacities, and
371 there was always retention even if the porosity was up to 50 times larger than the
372 beads diameter. This implies that for pre-filtration, in the view to NP quantification in a
373 real sample, the recoveries would be insufficient and very difficult to rationalize
374 because of the high particle heterogeneity (Gigault et al., 2021). We then looked for
375 an alternative to polymer-based membranes.

376 **Grid filtration**

377 Nylon single strand tissue and stainless-steel grids were tested (microscopic images
378 given in Figure SI 9). These two products did not present a multiple layer organization
379 and were promising in terms of high particles recoveries. The nylon single strand tissue
380 with a cut off at 1 or 5 µm retained between 20 to 30 % of the PSL whatever the particle
381 size tested (100, 350, 500, 750 and 1000 nm). The stainless-steel grids with cut off at

382 5 and 10 μm retained between 15 to 20 % with the same particles tested. As the
383 stainless-steel grid can be calcined in order to remove any impurities and be re-used,
384 we preferred its use to the nylon tissue.

385 **Dialysis, ultrafiltration**

386 In addition to gravimetric filtration, we also investigated PSL concentration changes
387 upon ultrafiltration and dialysis. The dialysis of PSCOOH-100 on 1 kDa PES
388 membranes led to important losses 50% after 48 hours and 70% after 72 hours. We
389 hypothesized that the particles certainly interacted with the membrane and remained
390 attached to it. We highly recommend controlling the particle concentrations after
391 proceeding to dialysis.

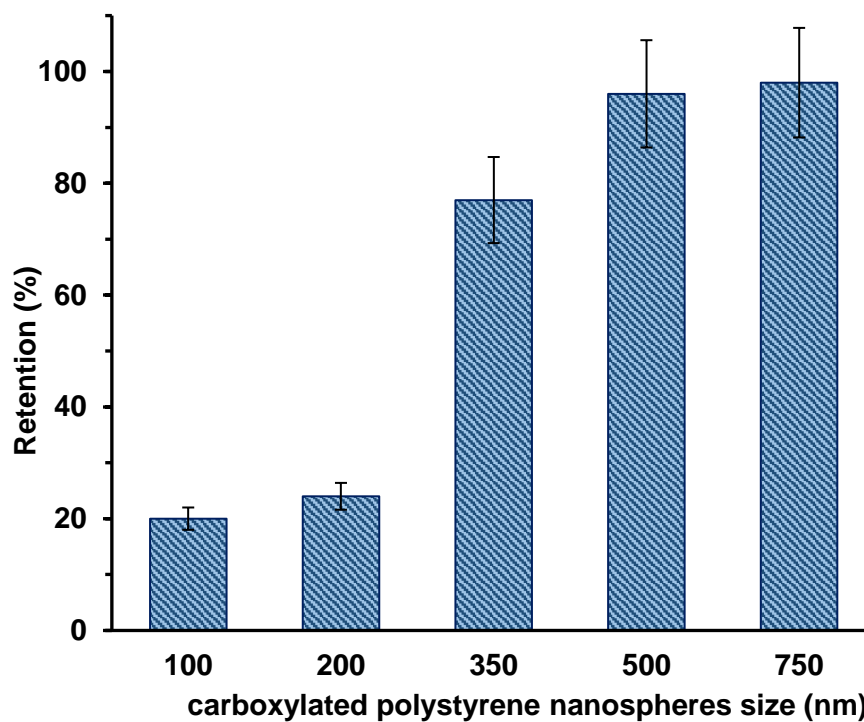
392 Another option to extract NP is the use of ultrafiltration (ter Halle et al., 2017). We
393 investigated the impact of ultrafiltration using a 50 kDa PES membrane and we
394 observed important losses: 40% with frontal filtration. Working with tangential
395 ultrafiltration slightly diminished this value to 35%. This is in agreement with previous
396 evaluations with 50% losses (Mintenig et al., 2018). As there are important PSL losses
397 after ultrafiltration we recommend to evaluate these losses is selecting this technic for
398 nanoplastic extraction.

399 **NP collection on glass fiber filters**

400 In order to analyze NP after the isolation step, it was proposed to deposit of a small
401 volume of the dispersion in a pyrolysis tube (Mintenig et al., 2018) or a pyrolysis cup
402 (Davranche et al., 2020; Wahl et al., 2021). But other options were described, like the
403 trapping of the particles on a filter. Sullivan et al. used PTFE filters as a medium for
404 particles introduction in the pyrolysis chamber (Sullivan et al., 2020). However, the
405 use of PTFE limits pyrolysis to 500°C. As an alternative the use of fiberglass filters was
406 proposed as a support to transfer plastic particles in the micrometric range (Albignac
407 et al., 2022; Leslie et al., 2022; Okoffo et al., 2020; Ribeiro et al., 2020). This is
408 promising because fiberglass filters can be calcined to remove any plastic residues
409 before use. Finally, it allows the use of higher pyrolysis temperatures compared to
410 PTFE which allow a better response for some polymers (Hermabessiere et al., 2018).
411 However, the retention capacity of the filters used to collect the NP was never
412 estimated.

413 We measured that calcined 0.7 μm glass fiber filters (GF/F, Whatman®) retained over
414 95% of PS-500, PS-750 and PS-1000. They retained 77% of the PS-350 and below
415 this size, the retention capacity was 20% (for PS-100 and PS-200; Figure 6 8). As a
416 conclusion, in the objective to collect nanoplastic, the retention capacity of glass fiber
417 filters was quantitative above 500 nm and below this size the retention capacity rapidly
418 diminishes with the particle sizes.

419



420

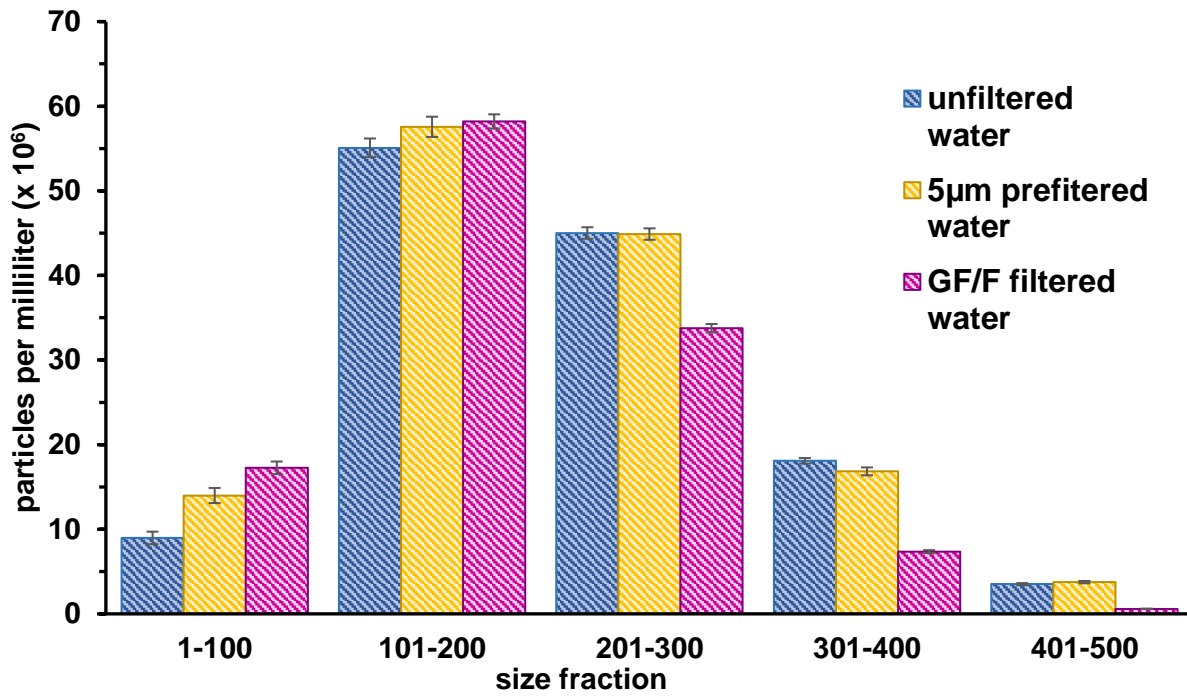
421 Figure 8: Retention of PSL carboxylated polystyrene nanospheres after filtration on
422 glass fiber filters (0.7 μm pore size). RSD were given with $n=3$.

423 **Application to river sampling**

424 As an illustration, 2 L of river water were processed with the two-step preparation
425 consisting in pre-filtration with a stainless-steel grid (5 μm cut-off) and collection with a
426 calcined glass fiber filter.

427 The colloidal content of the water was monitored by NTA. The pre-filtration of 2L
428 (Figure 9) shows that there is no alteration of the colloidal size distribution in the range
429 200-500 nm. Below 200 nm, after pre-filtration, there was a slight increase of the
430 particle abundance; this could be explained by shearing of the colloids and formation
431 of smaller particles in this class range. Above 500 nm the colloids present in the river

432 were not abundant enough to be monitored by NTA. We concluded that pre-filtration
433 on 5 μm steel grid did not alter much the particle size distribution in the nanometric
434 range – this is a promising way to recover quantitatively the colloidal fraction of a
435 natural sample.



436

437 Figure 9: NTA analysis of river water samples after pre-filtration on 5 μm inox grid and
438 filtration on glass fiber filter. RSD were given with n=3.

439 The second step consisted in filtration on glass fiber filters in order to trap NP. We
440 observed that 85% of the particles present in the river sample between 400-500 were
441 retained, 56% for 300-400 nm, and 25 % for 200-300 nm. Below 200 nm, it is more
442 difficult to interpret the results because the particles were more numerous after filtration
443 (Figure 9). Again, we assume that during filtration there is shearing of the colloids
444 resulting in more numerous smaller particles. The results with river water are less clear-
445 cut than with PSL because colloids are polydisperse and are made of aggregated
446 smaller particles that reorganize and rearrange easily under shear effect taking place
447 during filtration but we can conclude that above 400 nm more than 85 % of the particles
448 in the river sample were trapped in the glass fiber filter.

449

450 **Conclusion**

451 As a conclusion, we insist on the fact that sample preparation for nanoplastics analysis
452 is not yet established. Filtration (frontal or tangential ultrafiltration) or dialysis evaluated
453 here for nanoplastic retention capacities might be not the best option. These methods
454 present drawbacks but assets are also various. Among them, it remains a universal
455 method easily accessible to most research teams. It was important to demonstrate that
456 it can be used for such challenging topic, as long as enough precautions are taken and
457 yields of recoveries evaluated. In summary, this study demonstrates that there are
458 important interactions between PSL and polymer-based membrane during filtration;
459 important losses were recorded. Our recommendation would be to assess the retention
460 capacity of any membrane used whenever model NP are handled during tests. From
461 the perspective of quantifying NP in natural samples, the use of polymer-based
462 membranes as a pre-filtration step would result in important losses. As an alternative
463 we propose the use of stainless-steel grids because the losses were low with 20 %
464 retention capacity regardless the bead sizes tested. Filtration on the grid allow to
465 recover properly the colloidal fraction of the river water and alterations seemed
466 minimal. In a second step, in order to collect NP, the use of fiberglass filters is
467 promising because they present high retention capacities, and can be calcined to
468 remove any plastic residues before use. But the retention capacities of the glass fiber
469 filter, quantitative above 500 nm, rapidly decreased with the particle's diameter.

470 In conclusion, as very intense efforts are made for the technological development of
471 performant detection systems to reach smaller and smaller detection limits for NP
472 analysis (Schwaferts et al., 2019), extraction recoveries were systematically
473 overlooked. We recommend, in case of filtration before analysis that a well control
474 should be developed, along with the careful clean on the membrane. In order to provide
475 quantification, this step is absolutely necessary. The present paper proposes a method
476 to estimate the recovery rates during sample preparation and gives an illustration with
477 a river sample.

478

479 **Sample Credit author statement: Magali Albignac:** Investigation, Methodology,
480 Formal Analysis, Validation. **Emmanuelle Maria:** investigation, analysis, editing.
481 **Tiago de Oliveira:** investigation, analysis, editing. **Clement Roux:** Methodology,
482 Validation, Writing -reviewing and Editing. **Dominique Goudouneche:** electronic
483 microscopy measurements. **Anne-Françoise Mingotaud:** supervision, editing.
484 **Guillaume Bordeau:** Methodology, Validation, Writing -reviewing and Editing.
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494

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496

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