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1 **NANOPLASTICS : FROM MODEL MATERIALS TO COLLOIDAL**

2 **FATE**

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10

11 This review comes from a themed issue on Colloid Science for a Sustainable Future.

12

13 **Keywords.**

14 Nanoplastics, model materials, colloidal properties, stability, aggregation.

15

16 **Abstract**

17 Nanoplastics are pieces of plastic debris of mixed shape and chemical composition, which

18 can be present in the air, soil, freshwater bodies, seas, biota, and our diet. We know little

19 about the prevalence of nanoplastics and the risks they pose to the health of living

20 organisms; recent studies of plastics in the environment have not significantly contributed

21 information about this crucial topic. In this article, nanoplastics are presented as colloids.

22 Analysing their colloidal properties shows that their fate is governed by interfacial

23 properties, Trojan horse properties, and their stability in aqueous media. Their hazards,  
24 however, are as yet unknown.

25

26 ***Highlights.***

27 *A definition and model material for nanoplastics*

28 *Factors that govern the “Trojan horse properties” of nanoplastics*

29 *Why nanoplastics need to be considered with all their colloid characteristics*

30 *What drives nanoplastic stability and aggregation*

31

32 **Introduction**

33 The spread and potential risks of plastic debris is an international, global concern.  
34 Debris degrades in the environment into ever-smaller fragments, which increases the  
35 surface-to-volume ratio. The smallest pieces, microplastics and nanoplastics, are thought  
36 to affect ecosystem health and biodiversity conservation via multiple pathways [1,2]. It is  
37 known that debris size and risk to human health are inversely proportional, but there is not  
38 yet sufficient scientific knowledge of nanoplastics to establish relevant policies for risk  
39 assessment.

40 Due to the intrinsic properties of nanoplastics, especially in the aqueous phase where  
41 they behave as a colloid, research strategies (analytical methods, risk assessment, and  
42 sampling) need to be developed differently than for macroplastics and microplastics. The  
43 challenges increase as particle size decreases: nanoplastics interact more, or differently,  
44 with biological systems, and multiple research gaps must be adequately addressed to build  
45 a sustainable future.

46 Weathered macroplastics and microplastics from mismanaged waste are undoubtedly  
47 the most common source of nanoplastics, but material wear, such as of synthetic fabrics or  
48 car tire abrasion, can also lead to environmental nanoplastics [3]. The literature does not  
49 yet report any effective methods to trace the origin, source, or transport of microplastics,  
50 let alone nanoplastics. Finding such methods is challenging due to the diversity of the  
51 chemical makeup of the polymer matrices and the variety of additives that are used. Risk  
52 assessment for environmental microplastics and nanoplastics is challenging due to the lack  
53 of reference materials and validated detection methods, particularly in complex biological  
54 matrices.

55 Nanoplastics have been detected in seawater, but not quantified. It is known,  
56 however, that the smaller the plastics, the larger the quantities in terms of number of entities  
57 [4]. To invent new nanoplastic sampling and purification techniques, the scientific  
58 community is now using model materials of nanoplastic particles to establish, optimize,  
59 and apply novel analytical methods and sample preparation protocols to characterize and  
60 quantify nanoplastics in various matrices.

61 This review aims to present nanoplastics onto the context of plastic pollution,  
62 scientific issues associated to this specific kind of plastic debris from the model material  
63 to their behavior as a colloid. It starts by briefly recalling the plastic pollution context with  
64 the issues related to microplastics, and why we need a distinct research category for  
65 nanoplastics. We then consider problems in defining experimental conditions for  
66 nanoplastics. Nanoplastic model materials, with their interfacial and colloidal properties,  
67 are intrinsic to this research. The review finishes with the phenomena of homo- and  
68 heteroaggregation due to the colloidal and interfacial aspects of nanoplastics.

69

70 **From microplastics to nanoplastics: context and issues.**

71 Microplastics and nanoplastics have radically different chemical behaviours. The category  
72 of nanoplastics was defined recently in response to studies conducted on microplastics,  
73 which revealed that microplastics smaller than 1  $\mu\text{m}$  behaved so differently from larger  
74 microplastics (1  $\mu\text{m}$  to 5 mm) that they needed to be classified as a completely separate  
75 category [1,2,5]. Studies and reviews on microplastics have covered various environmental  
76 aspects [6–9] .

77

78 The scientific community classifies microplastics according to their origins, either as  
79 primary or secondary microplastics. Primary microplastics (PMPs) are plastic items that  
80 are deliberately manufactured at microscopic sizes (below 5 mm). These PMPs are used in  
81 exfoliating cosmetics, toothpaste, makeup, synthetic clothing, compressed air sandblasting  
82 processes, or as vectors of drugs in medicine [10]. This category also includes plastic  
83 granules of 2–5 mm diameter; often used as industrial raw material to generate plastic  
84 products [3]. Personal care products such as plastic microbeads are intentionally poured  
85 into wastewater, and are now banned in some countries to reduce this source of plastic  
86 pollution. Other PMPs found in the environment come from unintentional losses along the  
87 whole plastic value chain (weathering or leaching at open-air waste reception centers,  
88 accidental spills, losses during the transport of materials (plastic pellets) or through  
89 abrasion (such as tire wear on roads). PMPs can be deposited in rivers through sewage  
90 water (microfibre clothing fibres in washing machines) since wastewater treatment plants  
91 cannot separate them from wastewater. This is a way to reach another environmental

92 compartment since microplastics may also be concentrated in the sludge during the water  
93 treatment and used in many countries as agricultural fertilizer [11,12].

94 Secondary micro-plastics (SMPs) arise from mismanaged or dumped domestic and  
95 commercial plastic wastes, which are altered by physical, chemical, and biological  
96 processes in the environment, whether on soils or in surface water [12]. Exposure to  
97 sunlight, and in particular ultraviolet light, causes plastic photodegradation and surface  
98 oxidation, which may be followed by a thermo-oxidative process [13]. The degradation  
99 first occurs at the outer surface as revealed by a change of color (yellowing) and the  
100 appearance of microcracks as the surface becomes brittle [14]. SMPs will then be liable to  
101 fragment under the effects of abrasion, waves, and turbulence.

102 While the sample collection reported that macroplastics are almost exclusively composed  
103 of polyolefins with polyethylene (PE) / polypropylene (PP) with the ratio 80%/20%,  
104 microplastics collection exhibit a larger diversity. A microplastic collection in the north  
105 Atlantic subtropical gyre was composed of 73% PE, 13% PP, 8% PVC (polyvinylchloride),  
106 2% PS (polystyrene), and 1% PET (polyethylene terephthalate). This difference in term of  
107 composition versus the size of debris, may be attributed to several factors as the sample  
108 collection methods, in terms of items numbers where microplastics represent 80-90% of  
109 the collected items, the rest stands for meso- and macroplastics and in terms of the  
110 location (geographical and within the water column) where the collection has been done  
111 [14].

112 In the well-studied marine environment, the results of sampling campaigns in ocean gyres  
113 and models estimating the amount of microplastics differ, showing that only 1% of the  
114 expected quantity is observable [15]. Several hypotheses have been suggested to explain

115 this significant difference. The sampling method comes first; indeed, the regulatory mesh  
116 size of the plankton net is 330  $\mu\text{m}$ , lower mesh size down to 64  $\mu\text{m}$  has been reported for  
117 specific expeditions missing the smaller debris [16,17].

118 Consequently, the lower size limit for sampled microplastics varies between researchers  
119 due to the protocol [18]. The sampling procedure for microplastics in the water column  
120 misses the sinking fraction. As they were not properly sampled or even not sampled at all,  
121 sub-micron plastic particles were scarcely studied. Their quantification is still not possible  
122 as there are not yet any adequate detection tools.

123 Weathering of macroplastics and microplastics yields debris of sub-micron sizes [19–21]  
124 that are expected to be even more prevalent than their bulk counterparts. The mass and  
125 volume concentration of this small fraction dispersed in water is extremely low but  
126 incredibly high in terms of surface area as the surface area to the volume ratio gets smaller  
127 as the cell gets larger being proportional to  $1/D$ , with  $D$  the diameter. Up to now, this small  
128 category represents a low mass concentration that remains below the quantification limit  
129 of analytical methods, and the lack of withdrawal pre-concentration established procedures  
130 do not allow studies using "real, or even representative" items from surface water.  
131 Nevertheless, their presence was detected in the North Atlantic Gyre to prove their ability  
132 to accumulate in the oceans, just like macro and micro-plastics [22]. They also were  
133 detected in the sand [23] and soil [24] more recently. At last but not least, little work has  
134 been done so far onto the removal of nanoplastics. Due to the size of these debris,  
135 physical withdrawal is not feasible, however recent study reported interesting strategy  
136 using photocatalytic mineralization in aqueous media [25].

137

138 **Nanoplastics: a distinct category**

139 The size of plastic debris impacts their behaviour in the environment and their potential  
140 toxicity to living organisms. Nanoplastics are identified as emerging contaminants of  
141 concern [26]. Unlike larger particles, nanoplastics can exhibit colloidal behavior [1].  
142 Nanoplastics are generated by the degradation and fragmentation of larger plastic items in  
143 the environment, and should not be confused with engineered nanoparticles <100 nm [5].  
144 A recent review highlighted the role of the chemical composition, shape, and interface on  
145 the distinct fate of nanoplastics [27].  
146 Scientists, policymakers, and the public are becoming increasingly concerned about the  
147 prevalence of nanoplastics and the uncertainties surrounding their impacts, hazards, and  
148 risks to our environment and human health. Some of the evidence remains uncertain, and  
149 it is linked directly to their intrinsic nature: differences in polymer nature, item size  
150 (continuum size up to 1  $\mu\text{m}$ ), shape (spheroids, cylindrical pellets, fragments, flakes...),  
151 chemical additives (release or lixiviation), concentrations, measurements, fate.  
152 Nanoplastics should also be considered as a chemical cocktail coming from their  
153 production and environmental ageing. They may act as a pollutant carrier, as they have  
154 low-polarity surfaces that interact favourably with pollutant chemicals [28,29]. This may  
155 even be more pronounced with nanoplastics than microplastics due to a larger  
156 surface/volume ratio. The nanoplastic-pollutant cocktail may interact with and be ingested  
157 by organisms, and may either worsen or mitigate the pollutant exposure. The physical one  
158 should not be excluded from this potential chemical risk, such as inflammation, stress, and  
159 the gastrointestinal compartment [30].



160 The colloidal properties of nanoplastics are another critical factor in why they may have  
161 higher toxicities than microplastics [31]. As they are not affected by the density of their  
162 constituent plastic, they stay in the water column more easily, and may statistically have  
163 more interaction with pollutants and be carried by sea currents over long distances before  
164 being eventually ingested by marine organisms [32]. Nanoplastic behaviour and toxicity  
165 do not depend exclusively on size, and other properties should be evaluated for any risk  
166 assessment.

167

### 168 **Trojan horse properties of nanoplastics**

169 Virgin polymers, which are inert materials, are made suitable for commodity and specialty  
170 applications by the intentional addition of various additives to meet specific technical  
171 needs. The generic term “plastic” is defined by the International Union of Pure and Applied  
172 Chemistry as "a polymeric material that may contain other substances to improve  
173 performance and/or reduce costs" and should not be confused with the term “polymer”  
174 [33]. Plastic additives are divided into four groups [34]: 1) functional additives for  
175 additional properties (e.g., stabilizers, flame retardants, lubricants, foaming agents,  
176 biocides, slip agents, curing agents and plasticizers), 2) colorants (e.g. pigments, soluble  
177 azocolorants), 3) fillers: mostly inert, they increase the final product stiffness or hardness  
178 and reduce the material cost (e.g. mica, talc, kaolin, clay, calcium carbonate), and 4)  
179 reinforcements (e.g. carbon and glass fibres).

180 From a total of 1550 potential plastic additives, the European Chemical Agency (ECHA)  
181 identified more than 400 additives used for plastics in high volumes (*i.e.*, above 100  
182 tons/year) [35]. ECHA developed a model to calculate the release potential of the additives

183 and evaluated 155 of the 400 additives. This inventory is a starting point to decide which  
184 substances should be assessed as a matter of priority. Additives that are only incorporated  
185 by mixing into the matrix or chemically bonded to the polymer matrix should be considered  
186 separately [36] . The legislation is increasingly restrictive, and plastic additives, now as  
187 known as hazardous substances, are at least screened or regulated, and at best banned [37].  
188 Plastics have been discarded in the environment for decades, including those with  
189 persistent organic pollutant additives, and these plastics are now aging and degrading.  
190 Chemical residues (as monomers) or impurities are not within the scope of the definition  
191 of additive even though they should have the same behaviour and should be considered an  
192 additional potential risk. As mentioned above, nanoplastics are the plastic debris category  
193 that exhibits the highest surface-to-volume ratio. The release properties are linked to the  
194 diffusion of the additives into the plastic matrix and the partition of the additives from the  
195 matrix surface to the medium. As a result, additives embedded in debris are more easily  
196 released from nanoplastics than from larger plastic particles.  
197 Nanoplastics' high surface-to-volume ratios mean that they have statistically more contact  
198 with other chemical pollutants. Hydrophobic by nature, nanoplastics may behave as  
199 efficient sorbents, either by adsorption or absorption, for hydrophobic contaminants [29].  
200 This is an interfacial phenomenon called the "Trojan horse" property and may result in the  
201 transportation of eco-toxic molecules to release them in organisms. Concern about  
202 nanoplastics stems more from their potential pollutant carrier behaviour, rather than their  
203 intrinsic nature [28,29].  
204 Polychlorinated biphenyls (PCBs) [38] and polycyclic aromatic hydrocarbons (PAHs) [39]  
205 are the most cited persistent organic pollutants that bind to plastic debris. When adsorbed

206 onto nanoplastics, they can be transported along the water column in sediments, or  
207 assimilated by organisms, even if they are complexed with organic matter [11]. Surface  
208 coverage of nanoplastics by contaminants or biological species is called “corona  
209 formation” [27]. Beyond organic pollutants, plastics have a particular affinity with trace  
210 metals such as cadmium, lead, bromine [40–42] iron oxides [40], copper, zinc, cobalt,  
211 nickel, and arsenic [27,43]. Depending on the chemical properties of these substances, they  
212 may become bioavailable to organisms in the environment. Nanoplastics are dangerous due  
213 to their size, as they can penetrate natural barriers [44,45] to enter organisms’ bodies.

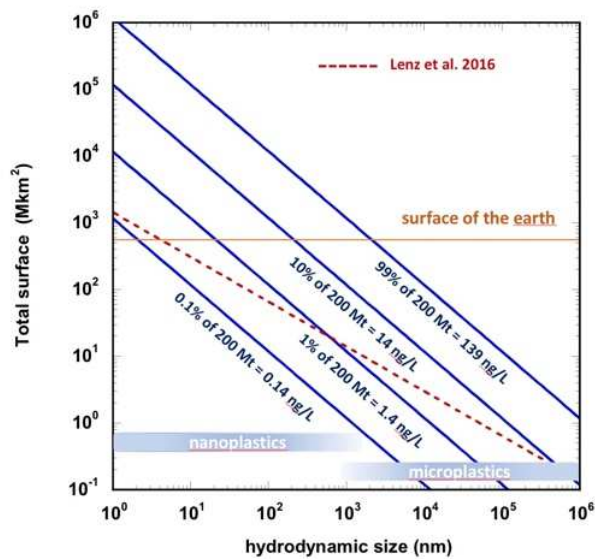
214

#### 215 **Nanoplastics and experimental conditions**

216 It is not yet possible to characterize and sample a sufficient quantity of nanoplastics in the  
217 environment. Most studies focus on the behaviour and toxicity of plastic nanospheres  
218 (PSLs) synthesized at the nanoscale, often commercially available. These PSLs have the  
219 advantage of being calibrated in size, known surface area, and, potentially, their known  
220 surface functionality. However, they may not be relevant to real environmental  
221 nanoplastics, as they are monodisperse, spherical, and do not have a comparable chemical  
222 formulation to manufactured plastics for industrial or domestic applications.

223 Even if we consider that the (eco)toxicological considerations of microplastics and  
224 nanoplastics are not in the scope of this paper, (eco)toxicological work and  
225 physicochemical studies still do need a relevant and standardized protocol, including the  
226 concentration range of nanoplastic-associated pollutants often present in a far different  
227 concentration than environmental conditions.

228 In the case of emerging contamination, it is always difficult to estimate an environmental  
229 concentration for performing toxicological tests. Nevertheless, nanoplastic environmental  
230 concentrations can be estimated by extrapolating from the known microplastic  
231 concentrations, a mass-conserving fragmentation process, and a theoretical 3D  
232 fragmentation process. The particulate concentration ( $n$ ) is then assumed to be proportional  
233 to-with the inverse of the particle diameter ( $d$ ) cubed:  $n \propto d^{-3}$  [46]. Following this hypothesis,  
234 Lenz et al. determined an exponent slightly lower than theoretically expected ( $n = 3888 \cdot d^{-2.67}$ ),  
235 which may be caused by size-dependent removal processes, lower dimensional  
236 breakdown (i.e., of flakes, sheets, and fibres), or considerable influence of new  
237 microplastic input of larger sizes. The lower accuracy in the detection of the smallest items  
238 in a sample must be considered. Based on the extrapolation proposed by Lenz, nanoplastics  
239 with a hydrodynamic size lower than  $1\mu\text{m}$  have a concentration of 0.14 to 1.4 ng/L (Figure  
240 1) in our oceans, which is about 0.1 to 1% of the 200 Mt of plastic waste already  
241 accumulated in the environment [47]. These concentration levels may appear low in terms  
242 of hazards (as a comparison, 4 ng/L represents half a grain of rice in an Olympic swimming  
243 pool). Nevertheless, due to their high nanoplastic surface-to-volume ratio, at this  
244 concentration and spread over the earth's surface water, the total surface of nanoplastics is  
245 similar to those of the earth.



246

247 *Figure 1. Total surface and concentration were calculated from different fractions of accumulated plastic waste (200Mt)*  
 248 *within our oceans ( $V = 1400 \cdot 10^6 \text{ km}^3$ ). The red dotted line is calculated from field data extrapolated by Lenz et al. using*  
 249 *a power-law regression ( $n = 3.188 \cdot d^{-2.67}$ ; with  $y$  as the particle concentration and  $x$  the particle size). The orange*  
 250 *straight line is the surface of the earth ( $357 \cdot 10^6 \text{ km}^2$ )*

251 Due to the Trojan horse properties, nanoplastics can transport various pollutants from the  
 252 environment into living organisms, contributing to toxin bioaccumulation [48]. This  
 253 behaviour is mainly governed by the nanoplastic interfacial properties regardless of their  
 254 aggregation status. Environmental and health issues may be strongly linked to a better  
 255 understanding of interfacial and colloidal properties of nanoplastics, which are related to  
 256 the characteristics (shape, size, functionality...) of the nanoplastics themselves [27].

257 Research groups have used various nanoplastics as model materials. It is difficult to  
 258 compare studies in a meaningful way without an official nanoplastic description, so work  
 259 is underway to establish criteria to properly define nanoplastics and microplastics,  
 260 especially for those used in toxicity and ecotoxicity studies [30].

261 Best practices to describe nanoparticles in the future will likely include: 1) reporting the  
 262 origin of nanoplastics; 2) describing them with shape, size, and size dispersion (followed

263 throughout the test), specific surface area, chemical nature (bulk, additives, impurities, and  
264 surface functionality); 3) reporting sample preparation methods (medium physicochemical  
265 properties, dispersion mode, nanoplastic concentration, nanoplastic stability within it); 4)  
266 identifying the presence/absence of ad/absorbed chemicals (either pollutant or tracer).

267 At present, producers rarely report the information listed above to describe the PSLs they  
268 make. Pikuda et al. were the first to demonstrate that the toxicity of commercial  
269 carboxylated PSLs (those commonly used in the research community) is due to additives  
270 such as sodium azide (bactericide), and not to the nanoplastics themselves [49].

271

#### 272 **Nanoplastic model materials**

273 Beyond commercial PSLs, "homemade" nanoplastic model materials have been developed  
274 by either the "bottom-up" or "top-down" approaches.

275 Nanoplastic model materials were obtained by the bottom-up approach by traditional  
276 colloidal chemistry methods such as conventional dispersed media (emulsions and  
277 nanoprecipitation). Designing such model materials directly in labs allows researchers to  
278 adapt the sample to the target study (analytical protocol development, (eco)toxicology with  
279 perfect knowledge of the concentration, size, chemical functionality, and content of  
280 additives and residuals).

281 Surfactants are needed for conventional colloidal chemistry (either emulsions or  
282 nanoprecipitation). As explained above, those may have detrimental effects on the  
283 behaviour studies by being themselves toxic to organisms, as has been shown for sodium  
284 dodecyl sulfate, Tween®, and Triton-X®. Organic solvents are also usually needed for  
285 nanoprecipitation and should be carefully removed or quantified [50]. It should be noted

286 using a green surfactant does not solve the toxicity issue; it is due to surfactants' intrinsic  
287 ability to adsorb at interfaces, bind to proteins, and solubilize components of membranes.  
288 To overcome this issue, some groups produce their own nanoplastic model materials by  
289 performing a so-called "soap-free emulsion" to form nanoplastics without surfactants or  
290 additives [51,52]. Following this strategy, Pessoni et al. were able to elaborate nanoplastic  
291 model materials free of emulsifiers or additives, including metals, and the model materials  
292 are negatively charged with modular properties [53].  
293 Furthermore, tagged nanoplastic model materials may be wanted as toxicology studies to  
294 evaluate their biological uptake or follow them in a specific environment [26]. Homemade  
295 isotopic [54] or metallic tracer embedded nanoplastics [55] have been described,  
296 fluorescently tagged model materials remain largely reported as Rhodamine or Nile red  
297 [56]. Nonetheless, it has been shown that fluorophores which may be toxic to organisms,  
298 could desorb from nanoplastics and be accumulated in the tissues of zebrafish larvae [57].  
299 At the same time, the nanoplastics themselves may be removed naturally by the body.  
300 Another approach to yield nanoplastic model materials mimicking the environmental ones  
301 leads to the reducing the scale of micro- and macro-plastic samples down to the nanoscale  
302 using top-down methods such as laser ablation [58], photodegradation [59], ultra-  
303 sonication [43], or mechanical degradation [60]. Polydisperse nanometric plastic model  
304 materials with an irregular shape, surface defects, and an oxidized surface have been  
305 obtained in these studies. However, getting large quantities of nanoplastics by these  
306 methods can be very difficult and time-consuming, and very few toxicity studies were  
307 made possible with these model materials.  
308

309 Table 1 reports the main characteristics of the different kinds of nanoplastics.

310 *Table 1. Main characteristics of nanoplastic categories as defined as being below 1 μm. PSLs and nanoplastic model*  
 311 *materials list the most common features reported within the literature. Real nanoplastic characteristics are given from*  
 312 *the current knowledge and perspective. (\*from this article list)*

Designation	Polystyrene latex (PSL)	Nanoplastic model materials			Real nanoplastics
Origin	Commercially available	Bottom-up approach	Top-down approach		To be sampled
Description	Polymer standard	From dispersed media	From primary plastic fragmentation)	From secondary plastic fragmentation	Environmental sample
Morphology	spherical	spherical	Flakes, irregularly shaped particles	flakes, irregularly shaped particles	flakes, irregularly shaped particles
Size dispersity	monodisperse	monodisperse	Continuum of size	Continuum of size	Continuum of size
Polymer nature	PS	PS	polyolefins, PS	Polyolefins, PS	Potentially any kind of plastics
Additive formula and content	From producer data	Known and controlled	From producer data	To be analyzed and determined	To be analyzed and determined
Ad/Absorbed pollutants	N/A	N/A	N/A	To be examined and determined	To be analyzed and determined
Surface functionality	Anionic, cationic, or none (qualitative information)	possibly known and adjustable	A brand new plastic	Potentially as oxidized plastic (aged debris)	Oxidized plastic (aged debris)
Embedded tracers	Fluorescent, metal	Fluorescent, metal	Fluorescent, metal	N/A	N/A
Key references*	and over 200 references within the literature	[50,53,55,61,62]	[58–60,63,64]	[60]	[22–24]

313

314 This research will advance more quickly once we have better knowledge of the nanoplastic  
 315 concentration in the medium, especially for the (eco)toxicity studies. When reported, really  
 316 high concentrations are often described.

317



318 **Colloidal stability of nanoplastics: homo and hetero-aggregation**

319 The stability of nanoparticles, mainly governed by the size and shape of the nanoplastics,  
320 is a determining factor in their bioavailability and toxicity [5]. The majority of  
321 ecotoxicological studies do not verify the stability of PSLs in the experimental exposure  
322 medium. In freshwater studies this does not have a significant impact on the results,  
323 because nanoplastics are often stable in freshwater environments. In seawater studies,  
324 however, plastic particle size is an essential factor because significant ionic forces cause  
325 nanoplastic aggregation [11,65].

326 The stability of plastic nanoparticles in the experimental exposure medium has rarely been  
327 studied so far. Several studies have measured the hydrodynamic diameter of nanoplastic  
328 aggregates in the experimental conditions without specifying measurement time. However,  
329 aggregation kinetics allows researchers to define the size characteristics of the particle that  
330 will ultimately interact with organisms. When the particles aggregate, the size of the  
331 agglomerates increases, and their number decreases accordingly. Changes in these  
332 parameters have a considerable impact on the experimental conditions of organisms and,  
333 therefore, on the interpretations that may result from them.

334 As explained in the review of Hotze et al. for classical colloids and nanoparticles [66],  
335 nanoplastics, due to their high surface reactivity, interact with natural and anthropogenic  
336 colloids such as metallic oxides and other organic molecules and inorganic compounds  
337 (clay, silica, etc.) as soon as they diffuse in the environment. These interactions induce  
338 hetero-aggregation of nanoplastics according to the surrounding media and the  
339 environmental context. The final hetero-aggregate form, size, and composition drive the  
340 final behaviour and impact as much as their size classes and aggregation behavior is linked

341 to the environmental features (pH, salinity, sunlight, etc.). Hetero-aggregation, in which  
342 two or more different types of particles form aggregates, is more likely to occur than  
343 homoaggregation for nanoplastics due to the much higher number of natural colloids.

344 However, homoaggregation studied at the laboratory scale is still very useful to  
345 characterize the stability of nanoplastics as a function of their physicochemical properties  
346 and the salinity of the medium. At the ppm range of concentrations, the critical coagulation  
347 concentration (CCC) can be determined by dynamic or static light scattering and  
348 UV/visible spectroscopy. These methods have been used in recent years to study the  
349 stability of nanoplastics, and of all the existing methods [67], the time-resolved DLS is the  
350 most common.

351 Table 1 summarizes recent laboratory studies investigating homoaggregation rates and  
352 CCCs of anionic-modified nanoplastics based on PS, PET, and PE. In general, the data  
353 show that the particle–particle attachment efficiency increases with the ionic strength; this  
354 is due to the compression of the electrical double layer, in agreement with the Deryaguin–  
355 Landau–Verwey–Overbeek (DLVO) theory. The importance of valence is shown in Table  
356 1, where divalent cations ( $\text{Ca}^{2+}$ ) destabilize nanoplastics at lower concentrations than  
357 monovalent cations ( $\text{Na}^+$ ), following the Schulze–Hardy Rule ( $\text{CCC} \propto 1/z^n$ , with  $2 < n < 6$ )  
358 [68].

359 At the same pH and for an equivalent size, the large spread of the CCC data can be  
360 explained by the nanoplastics' commercial origin. Manufacturers mainly use surfactants  
361 and are often unwilling to inform the end user even that surfactants were employed and  
362 how much remained in the latex dispersion, or about details of the surface charge density.  
363 Nonionic surfactants attach covalently to the nanoparticle surface, and neither extensive

364 dialysis nor ion exchange resins will remove them. There is usually significant batch-to-  
365 batch variability and differences between manufacturers, as well, which inevitably leads to  
366 differences in CCC experimental data.

367 Compared with commercial nanoplastics, those obtained from laser ablation or mechanical  
368 grinding are polydisperse with irregular shapes and complex surface chemistry, and they  
369 are free from residual surfactants. Thus, they are more suitable model materials to study  
370 the aggregation of nanoplastics in the environment. Recently, Veclin et al. employed  
371 nanoplastics free of surfactant and chemical control of the surface to model material surface  
372 weathering [69]. The samples presented a wide distribution of relevant surface properties  
373 such as functionality (ionizable carboxylic group, 0.10 to 1.7 mmol g<sup>-1</sup>), hydrophobicity  
374 (surface energy, 2.20 to 37.5 mJm<sup>-2</sup>), surface morphology (smooth or "raspberry-  
375 textured"), zeta potential (-31 to -21 mV) and anisotropy in shape. The CCC  
376 measurements demonstrate that spherical nanoplastics are more stable in seawater (CCC >  
377 600 mmol L<sup>-1</sup>) than anisotropic nanoplastics (CCC ~ 100 mmol L<sup>-1</sup>) are.

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394 *Table 2. Review of recent studies reporting the homoaggregation rates and critical coagulation*  
 395 *concentrations (CCCs) in mono- and divalent electrolytes of anionic surface-functionalized nanoplastic*  
 396 *(not chemically specified but with negative zeta potential, carboxylated, sulfonic and formic. The origin of*  
 397 *the nanoplastics is commercial (emulsion), from laser ablation, mechanical fragmentation, U.V.*  
 398 *irradiation, and filtration methods. \* given with the plastic nature (PS, PE, PET) and the surface chemical*  
 399 *function (none, COOH, SO<sub>3</sub>H, C<sub>2</sub>H<sub>2</sub>O) for the commercial nanoplastics (PSLs) or the elaboration methods,*  
 400 *namely grinded, U.V., laser, filt.*

nanoplastic*	Size (nm)	CCC (mmol/L)		pH	Method	Origin	Ref		
		NaCl	CaCl <sub>2</sub>						
PSL	240	140	25	6	DLS	commercial	[70]		
		450	33	6			[71]		
		300-500	--	3 to 10			[71]		
	100	460	32	6			[72]		
		591	71	7.5			[73]		
		135	--	6			[74]		
		500	40	5 to 9			SLS	commercial +	[75]
	93	310	29	u			DLS	commercial	[76]
	55	950	--	6					[74]
	50	264	29	5					[77]
PSL-COOH	110	--	10	u	DSL	commercial	[78]		
	97	308	28	u		chemical surface	[76]		
	50	191	16	5		commercial	[77]		
	28	800	10	5		[79]			
PSL-SO <sub>3</sub> H	90	159	12	6	DLS	commercial	[80]		
	80	264	29	5	[77]				
PSL-C <sub>2</sub> H <sub>2</sub> O	50	84	10	5	DLS	commercial	[77]		
PS-grinded	350	59	--	6.5	DLS	mechanical	[81]		
		67		8		degradation	[81]		
PS-UV	100	957-1108	--	u	DLS	UV-irradiation in H <sub>2</sub> O <sub>2</sub> – 60 to 120h	[73]		
						[73]			
	50- 100	760-458	27-8	6		UV-irradiation 1 to 24h	[71]		
	50	280-1300	--	3-10		UV-irradiation 24h	[71]		
PET-laser	110	700		u	U.V. /Vis	Ablation laser	[58]		
PET-grinded	300- 700	54	2.1	6	DLS	mechanical	[82]		
		110	5,6	10		degradation	[82]		
PE-filt	200	80	0.1	5	DLS	filtration of PE microplastic stabilized with SDS (0.1%)	[79]		

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402 The size, shape, and surface characteristics of any nanoplastic model material will affect  
403 the heteroaggregation phenomenon and, therefore, its stability in the environment.

404 As the first test of heteroaggregation, Cai *et al.* used fluorescent 100 nm commercial  
405 polystyrene standards (PSLs) with  $\text{CaCl}_2$ ,  $\text{NaCl}$ , and  $\text{FeCl}_2$  in the presence of humic  
406 substances[83]. They demonstrated that with an inorganic heterogeneity such as Fe(III) in  
407 the hetero-aggregation, humic substances play the role of a stabilizing agent of PSLs.

408 Orikhova *et al.* came to the same conclusion when studying commercial 20 nm PSL-NH<sub>2</sub>  
409 in the presence of  $\text{Fe}_2\text{O}_3$  and alginate as model organic matter. Due to the amidine function,  
410 the ratio of the concentration of natural organic matter *over* PSL-NH<sub>2</sub> affects the stability  
411 of the hetero-aggregates in fresh water [84]. The amidine group was also used by Wu *et al.*  
412 using PSL-NH<sub>2</sub> in humic acid to investigate the formation of hetero-aggregate and their  
413 impact on *Daphnia magna* [85]. These authors demonstrated that the positively charged  
414 nanoparticles of polystyrene induced more toxicity that was tempered by the presence of  
415 humic acid. Tallec *et al.* came to another conclusion, which was that humic acid did not  
416 affect the aggregation and stability of PSL-NH<sub>2</sub> compared to PSL-COOH as observed by  
417 Orikhova [84,86]. This can be explained by the presence of Tween20® residue in the  
418 standards used by Tallec *et al.* As previously described, the presence of an undeclared  
419 surfactant or another stabilizing agent in commercial nanoplastic model materials, such as  
420 PSLs, induces a change in the hetero-aggregation formation as observed for toxicological  
421 studies [49].

422 For negatively charged nanoplastics, the aggregation of PS, PET, or PE nanoplastics was  
423 significantly reduced with organic matter, and the CCC value increased substantially in  
424 NaCl solution. Pradel *et al.* showed that two types of natural organic matter (humic acid

425 and alginate) enhanced the aqueous stability of anisotropic nanoplastics over time at high  
426 ionic strengths. Depending on the organic matter's nature, different stabilizing mechanisms  
427 were revealed using dynamic light scattering and asymmetrical flow field flow  
428 fractionation coupled to static light scattering [81].

429 Humic acid provides electrostatic repulsion between particles, and some larger humic acid  
430 molecules causes a steric hindrance. Sodium alginate sorbs onto and separates particles and  
431 small aggregates of nanoplastics. The covered particles are stabilized by steric hindrance.  
432 However, in divalent electrolytes, Yu *et al.* described the enhanced stability of PSL-COOH  
433 at low concentrations of Suwannee river natural organic matters (SRNOM) and altered  
434 stability at high concentrations of SRNOM due to the interparticle bridging effect caused  
435 by the  $\text{Ca}^{2+}$  and carboxyl groups of SRNOM [76]. The cation bridging effect was also  
436 observed for anisotropic nanoplastics (PS-laser) in the presence of high concentrations of  
437 divalent electrolytes and SRNOM [76]. Similar results were shown by Singh *et al.*, who  
438 noted faster aggregation with  $\text{CaCl}_2$  due to complexation with the dissolved organic matter  
439 [70]. Otherwise, the mineral clay colloids participate in hetero-aggregation with  
440 nanoplastics under the influence of salts. The stability of nanoplastic colloidal suspensions  
441 decreased in the presence of the positively charged goethite or magnetite, while it was  
442 affected by the negatively charged montmorillonite and kaolinite, suggesting that there was  
443 a strong electrostatic attraction between nanoplastics and the two iron oxides [87]. To  
444 complexify the solution chemistry, Li *et al.* have shown that the hetero-aggregation of  
445 nanoplastics with metal-based nanoparticles (e.g.,  $\text{CeO}_2$ -nanoparticle of 25 nm) and humic  
446 acid was influenced by the nanoplastic surface chemical functionality ( $\text{COOH}$ ,  $\text{SO}_3\text{H}$ ,  
447  $\text{C}_2\text{H}_2\text{O}$ ,  $\text{NH}_2$ ), the pH, and the presence of a divalent electrolyte ( $\text{CaCl}_2$ ) [77].

448 Some authors recently started to consider more relevant organic matter for marine systems,  
449 such as exopolymeric substances (EPS) from bacteria in the marine system. These EPS are  
450 polysaccharide macromolecules and present structures susceptible to interacting  
451 preferentially with nanoplastics through hydrophobic attraction even if the PSL used had  
452 surfactant traces [88]. Grassi *et al.* have analyzed the dynamic formation of an eco-corona  
453 coming from the interaction of PSL-COOH with EPS produced by the marine diatom *P.*  
454 *tricornutum* [89]. The chemical characterization revealed a predominance of  
455 polysaccharide material concerning proteins over a broad molecular weight range. The  
456 addition of EPS significantly reduces PSL-COOH aggregation in seawater [89].

457 The authors of this review attempted to consolidate the studies on the hetero-aggregation  
458 of nanoplastics, and to investigate the surface influence on the hetero-aggregation  
459 formation. However, bias and lack of coherence on parameters such as 1) the functional  
460 group, 2) the size and shape of the model material, 3) the composition, and 4) the evaluation  
461 of the hetero-aggregation of nanoplastics made it difficult to complete our review. The  
462 hetero-aggregation and the final structure of the aggregates is a crucial piece to understand,  
463 as it will define nanoplastics' fate and impact. The influence of the functional group of the  
464 model material on the hetero-aggregation formation comes first. In the environment, the  
465 colloidal stability of the nanoplastics is governed by the organic matter sorbed and/or  
466 associated with it. Therefore, the surface charge and functionality does not influence its  
467 reactivity with natural media due to the corona of the natural organic matter and its  
468 specificities.

469 While it is intuitively accepted that nanoplastics result from the degradation of plastic  
470 fragments in seawater or on beaches, it is also possible that this degradation takes place in

471 rivers [90]. However, surface and groundwater are not a continuous phase and are made up  
472 of several systems that can transport, trap or re-suspend nanoparticles. These phenomena  
473 lead to a crucial question: what is the impact of this transport on the behaviour of  
474 nanoplastics? As described above, physicochemical studies of colloids show that  
475 nanoplastic aggregation depends on the ionic strength of the dispersion medium. These  
476 experiments do not consider the spatiotemporal dynamics of the medium's  
477 physicochemical properties, including the flux and ionic strength gradients. However, the  
478 particles, when in fresh surface water, will transit through gradients of significant ionic  
479 strength before reaching ocean water bodies, such as estuaries, lagoons, and mangroves  
480 [91].

481 Likewise, most ecotoxicological studies have not considered the complexity of natural  
482 aquatic environments either. The procedure is similar from one study to another: the PSLs  
483 (often at high concentrations) are dispersed in MilliQ water. This dispersion is then spread  
484 to the experimental exposure medium to obtain a given final concentration while handling  
485 a minimum sample volume. While this procedure makes sense for liquid contaminants, it  
486 is inappropriate for nanoplastics. The particles concentrated in freshwater are then  
487 suddenly re-dispersed in a medium with a distinct ionic strength. It should be added that  
488 the experimental conditions are favorable to aggregation phenomena, and the observed  
489 effects may be mistakenly attributed to nanoplastics while they are due to their  
490 agglomerates. As a result, it is possible that ecotoxicology studies underestimate, or poorly  
491 estimate, the effects of nanoplastics.

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494 **Conclusion**

495 The scientific challenge that remains is to determine the behaviour of nanoplastics in  
496 aquatic environments, at the freshwater-saltwater and liquid-ice interfaces, which are  
497 essential areas for biodiversity. The other challenge will be to understand this  
498 environmental behaviour, and the mechanisms of action, and to determine whether  
499 nanoplastics are toxic.

500 However, due to the lack of feasible technologies, nanoplastics have not been yet sampled  
501 or quantified in the environment and the research needs material models. It should be  
502 mentioned that just as a single nanoplastic particle does not exist, a universal nanoplastic  
503 model material couldn't be claimed. However, a material become a relevant model as soon  
504 as its properties are properly controlled, known and reported. As shown, within influent  
505 properties, colloidal behavior is of previous importance since it involves not only the  
506 stability of the nanoplastic but also will help to understand its bioavailability.

507

508 **References**

509 Papers of particular interest, published within the period of review, have been highlighted  
510 as:

511 \* of special interest

512 \*\* of outstanding interest+

513 [1] Gigault J, Halle AT, Baudrimont M, Pascal P-Y, Gauffre F, Phi T-L, et al.

514 Current opinion: What is a nanoplastic? *Environ Pollut* 2018;235:1030–4.

515 <https://doi.org/10.1016/j.envpol.2018.01.024>.

516 [2] Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard

517 AE, et al. Are We Speaking the Same Language? Recommendations for a Definition and

518 Categorization Framework for Plastic Debris. *Environ Sci Technol* 2019;53:1039–47.

519 <https://doi.org/10.1021/acs.est.8b05297>.

520 [3] Boucher J, Friot D. Primary microplastics in the oceans: A global evaluation of

521 sources. IUCN International Union for Conservation of Nature; 2017.  
522 <https://doi.org/10.2305/IUCN.CH.2017.01.en>.

523 [4] Phuong NN, Poirier L, Lagarde F, Kamari A, Zalouk-Vergnoux A. Microplastic  
524 abundance and characteristics in French Atlantic coastal sediments using a new  
525 extraction method. *Environ Pollut* 2018;243:228–37.  
526 <https://doi.org/10.1016/j.envpol.2018.08.032>.

527 [5] Gigault J, El Hadri H, Nguyen B, Grassl B, Roweczyk L, Tufenkji N, et al.  
528 Nanoplastics are neither microplastics nor engineered nanoparticles. *Nat Nanotechnol*  
529 2021;1–7. <https://doi.org/10.1038/s41565-021-00886-4>.

530 [6] Campos da Rocha FO, Martinez ST, Campos VP, da Rocha GO, de Andrade JB.  
531 Microplastic pollution in Southern Atlantic marine waters: Review of current trends,  
532 sources, and perspectives. *Sci Total Environ* 2021;782:146541.  
533 <https://doi.org/10.1016/j.scitotenv.2021.146541>.

534 [7] Harris PT. The fate of microplastic in marine sedimentary environments: A  
535 review and synthesis. *Mar Pollut Bull* 2020;158:111398.  
536 <https://doi.org/10.1016/j.marpolbul.2020.111398>.

537 [8] Rillig MC, Lehmann A. Microplastic in terrestrial ecosystems. *Science*  
538 2020;368:1430–1. <https://doi.org/10.1126/science.abb5979>.

539 [9] Zhang Y, Kang S, Allen S, Allen D, Gao T, Sillanpää M. Atmospheric  
540 microplastics: A review on current status and perspectives. *Earth-Sci Rev*  
541 2020;203:103118. <https://doi.org/10.1016/j.earscirev.2020.103118>.

542 [10] Auta HS, Emenike CU, Fauziah SH. Distribution and importance of microplastics  
543 in the marine environment: A review of the sources, fate, effects, and potential solutions.  
544 *Environ Int* 2017;102:165–76. <https://doi.org/10.1016/j.envint.2017.02.013>.

545 [11] Alimi OS, Farnier Budarz J, Hernandez LM, Tufenkji N. Microplastics and  
546 Nanoplastics in Aquatic Environments: Aggregation, Deposition, and Enhanced  
547 Contaminant Transport. *Environ Sci Technol* 2018;52:1704–24.  
548 <https://doi.org/10.1021/acs.est.7b05559>.

549 [12] Cole M, Lindeque P, Halsband C, Galloway TS. Microplastics as contaminants in  
550 the marine environment: A review. *Mar Pollut Bull* 2011;62:2588–97.  
551 <https://doi.org/10.1016/j.marpolbul.2011.09.025>.

552 [13] Andrady AL. Microplastics in the marine environment. *Mar Pollut Bull*  
553 2011;62:1596–605. <https://doi.org/10.1016/j.marpolbul.2011.05.030>.

554 [14] ter Halle A, Ladirat L, Gendre X, Goudouneche D, Pusineri C, Routaboul C, et al.  
555 Understanding the Fragmentation Pattern of Marine Plastic Debris. *Environ Sci Technol*  
556 2016;50:5668–75. <https://doi.org/10.1021/acs.est.6b00594>.

557 [15] Cózar A, Echevarría F, González-Gordillo JI, Irigoien X, Úbeda B, Hernández-  
558 León S, et al. Plastic debris in the open ocean. *Proc Natl Acad Sci* 2014;111:10239–44.  
559 <https://doi.org/10.1073/pnas.1314705111>.

560 [16] Figueiredo GM, Vianna TMP. Suspended microplastics in a highly polluted bay:  
561 Abundance, size, and availability for mesozooplankton. *Mar Pollut Bull* 2018;135:256–  
562 65. <https://doi.org/10.1016/j.marpolbul.2018.07.020>.

563 [17] Mattsson K, Hansson L-A, Cedervall T. Nano-plastics in the aquatic environment.  
564 *Environ Sci: Processes Impacts* 2015;17:1712–21. <https://doi.org/10.1039/C5EM00227C>.

565 [18] Frias JPGL, Nash R. Microplastics: Finding a consensus on the definition. *Mar*  
566 *Pollut Bull* 2019;138:145–7. <https://doi.org/10.1016/j.marpolbul.2018.11.022>.

- 567 [19] Gigault J, Pedrono B, Maxit B, Halle AT. Marine plastic litter: the unanalyzed  
568 nano-fraction. *Environ Sci: Nano* 2016;3:346–50. <https://doi.org/10.1039/C6EN00008H>.
- 569 [20] Lambert S, Wagner M. Characterisation of nanoplastics during the degradation of  
570 polystyrene. *Chemosphere* 2016;145:265–8.  
571 <https://doi.org/10.1016/j.chemosphere.2015.11.078>.
- 572 [21] Lambert S, Wagner M. Formation of microscopic particles during the degradation  
573 of different polymers. *Chemosphere* 2016;161:510–7.  
574 <https://doi.org/10.1016/j.chemosphere.2016.07.042>.
- 575 [22] Ter Halle A, Jeanneau L, Martignac M, Jardé E, Pedrono B, Brach L, et al.  
576 Nanoplastic in the North Atlantic Subtropical Gyre. *Environ Sci Technol*  
577 2017;51:13689–97. <https://doi.org/10.1021/acs.est.7b03667>.
- 578 [23] Davranche M, Lory C, Juge CL, Blancho F, Dia A, Grassl B, et al. Nanoplastics  
579 on the coast exposed to the North Atlantic Gyre: Evidence and traceability. *NanoImpact*  
580 2020;20. <https://doi.org/10.1016/j.impact.2020.100262>.
- 581 [24] Wahl A, Le Juge C, Davranche M, El Hadri H, Grassl B, Reynaud S, et al.  
582 Nanoplastic occurrence in a soil amended with plastic debris. *Chemosphere* 2021;262.  
583 <https://doi.org/10.1016/j.chemosphere.2020.127784>.
- 584 [25] Allé PH, Garcia-Muñoz P, Adouby K, Keller N, Robert D. Efficient  
585 photocatalytic mineralization of polymethylmethacrylate and polystyrene nanoplastics by  
586 TiO<sub>2</sub>/β-SiC alveolar foams. *Environ Chem Lett* 2020. [https://doi.org/10.1007/s10311-](https://doi.org/10.1007/s10311-020-01099-2)  
587 020-01099-2.
- 588 [26] Koelmans AA. Proxies for nanoplastic. *Nat Nanotechnol* 2019;14:307–8.  
589 <https://doi.org/10.1038/s41565-019-0416-z>.
- 590 [27] Kihara S, Köper I, Mata JP, McGillivray DJ. Reviewing nanoplastic toxicology:  
591 It's an interface problem. *Adv Colloid Interface Sci* 2021;288:102337.  
592 <https://doi.org/10.1016/j.cis.2020.102337>.
- 593 [28] Mitrano DM, Wick P, Nowack B. Placing nanoplastics in the context of global  
594 plastic pollution. *Nat Nanotechnol* 2021;16:491–500. [https://doi.org/10.1038/s41565-](https://doi.org/10.1038/s41565-021-00888-2)  
595 021-00888-2.
- 596 [29] Hartmann NB, Rist S, Bodin J, Jensen LH, Schmidt SN, Mayer P, et al.  
597 Microplastics as vectors for environmental contaminants: Exploring sorption, desorption,  
598 and transfer to biota. *Integr Environ Assess Manag* 2017;13:488–93.  
599 <https://doi.org/10.1002/ieam.1904>.
- 600 [30] Kokalj AJ. Quality of nanoplastics and microplastics ecotoxicity studies: Refining  
601 quality criteria for nanomaterial studies. *J Hazard Mater* 2021;12.
- 602 [31] Bouwmeester H, Hollman PCH, Peters RJB. Potential Health Impact of  
603 Environmentally Released Micro- and Nanoplastics in the Human Food Production  
604 Chain: Experiences from Nanotoxicology. *Environ Sci Technol* 2015;49:8932–47.  
605 <https://doi.org/10.1021/acs.est.5b01090>.
- 606 [32] Maity S, Pramanick K. Perspectives and challenges of micro/nanoplastics-induced  
607 toxicity with special reference to phytotoxicity. *Glob Change Biol* 2020;26:3241–50.  
608 <https://doi.org/10.1111/gcb.15074>.
- 609 [33] Vert M, Doi Y, Hellwich K-H, Hess M, Hodge P, Kubisa P, et al. Terminology  
610 for biorelated polymers and applications (IUPAC Recommendations 2012). *Pure Appl*  
611 *Chem* 2012;84:377–410. <https://doi.org/10.1351/PAC-REC-10-12-04>.
- 612 [34] Hahladakis JN, Velis CA, Weber R, Iacovidou E, Purnell P. An overview of

613 chemical additives present in plastics: Migration, release, fate and environmental impact  
614 during their use, disposal and recycling. *J Hazard Mater* 2018;344:179–99.  
615 <https://doi.org/10.1016/j.jhazmat.2017.10.014>.

616 [35] Plastic additives initiative - ECHA n.d. [https://echa.europa.eu/plastic-additives-](https://echa.europa.eu/plastic-additives-initiative)  
617 initiative (accessed April 25, 2021).

618 [36] Wagner S, Schlummer M. Legacy additives in a circular economy of plastics:  
619 Current dilemma, policy analysis, and emerging countermeasures. *Resour Conserv*  
620 *Recycl* 2020;158:104800. <https://doi.org/10.1016/j.resconrec.2020.104800>.

621 [37] Technical Guidelines n.d.  
622 [http://www.basel.int/Implementation/POPsWastes/TechnicalGuidelines/tabid/5052/Defau](http://www.basel.int/Implementation/POPsWastes/TechnicalGuidelines/tabid/5052/Default.aspx)  
623 [lt.aspx](http://www.basel.int/Implementation/POPsWastes/TechnicalGuidelines/tabid/5052/Default.aspx) (accessed April 25, 2021).

624 [38] Velzeboer I, Kwadijk CJAF, Koelmans AA. Strong sorption of PCBs to  
625 nanoplastics, microplastics, carbon nanotubes, and fullerenes. *Environ Sci Technol*  
626 2014;48:4869–76. <https://doi.org/10.1021/es405721v>.

627 [39] Lee H, Shim WJ, Kwon J-H. Sorption capacity of plastic debris for hydrophobic  
628 organic chemicals. *Sci Total Environ* 2014;470–471:1545–52.  
629 <https://doi.org/10.1016/j.scitotenv.2013.08.023>.

630 [40] Davranche M, Veclin C, Pierson-Wickmann A-C, El Hadri H, Grassl B,  
631 Rowenczyk L, et al. Are nanoplastics able to bind significant amount of metals? The lead  
632 example. *Environ Pollut* 2019;249:940–8. <https://doi.org/10.1016/j.envpol.2019.03.087>.

633 [41] Holmes LA, Turner A, Thompson RC. Adsorption of trace metals to plastic resin  
634 pellets in the marine environment. *Environ Pollut* 2012;160:42–8.  
635 <https://doi.org/10.1016/j.envpol.2011.08.052>.

636 [42] Massos A, Turner A. Cadmium, lead and bromine in beached microplastics.  
637 *Environ Pollut* 2017;227:139–45. <https://doi.org/10.1016/j.envpol.2017.04.034>.

638 [43] Baudrimont M, Arini A, Guégan C, Venel Z, Gigault J, Pedrono B, et al.  
639 Ecotoxicity of polyethylene nanoplastics from the North Atlantic oceanic gyre on  
640 freshwater and marine organisms (microalgae and filter-feeding bivalves). *Environ Sci*  
641 *Pollut Res* 2019. <https://doi.org/10.1007/s11356-019-04668-3>.

642 [44] Galloway TS, Dogra Y, Garrett N, Rowe D, Tyler CR, Moger J, et al.  
643 Ecotoxicological assessment of nanoparticle-containing acrylic copolymer dispersions in  
644 fairy shrimp and zebrafish embryos. *Environ Sci Nano* 2017;4:1981–97.  
645 <https://doi.org/10.1039/C7EN00385D>.

646 [45] Triebkorn R, Braunbeck T, Grummt T, Hanslik L, Huppertsberg S, Jekel M, et  
647 al. Relevance of nano- and microplastics for freshwater ecosystems: A critical review.  
648 *TrAC - Trends Anal Chem* 2019;110:375–92. <https://doi.org/10.1016/j.trac.2018.11.023>.

649 [46] Lenz R, Enders K, Nielsen TG. Microplastic exposure studies should be  
650 environmentally realistic. *Proc Natl Acad Sci* 2016;113:E4121–2.  
651 <https://doi.org/10.1073/pnas.1606615113>.

652 [47] Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, et al.  
653 Plastic waste inputs from land into the ocean. *Science* 2015;347:768–71.  
654 <https://doi.org/10.1126/science.1260352>.

655 [48] Stapleton PA. Toxicological considerations of nano-sized plastics. *AIMS Environ*  
656 *Sci* 2019;6:367–78. <https://doi.org/10.3934/environsci.2019.5.367>.

657 [49] Pikuda O, Xu EG, Berk D, Tufenkji N. Toxicity Assessments of Micro- and  
658 Nanoplastics Can Be Confounded by Preservatives in Commercial Formulations. *Environ*

659 Sci Technol Lett 2018;6:21–5. <https://doi.org/10.1021/acs.estlett.8b00614>.

660 [50] Balakrishnan G, Déniel M, Nicolai T, Chassenieux C, Lagarde F. Towards more  
661 realistic reference microplastics and nanoplastics: preparation of polyethylene  
662 micro/nanoparticles with a biosurfactant. *Environ Sci Nano* 2019;6:315–24.  
663 <https://doi.org/10.1039/C8EN01005F>.

664 [51] Kihara S, Van Der Heijden NJ, Seal CK, Mata JP, Whitten AE, Köper I, et al.  
665 Soft and Hard Interactions between Polystyrene Nanoplastics and Human Serum  
666 Albumin Protein Corona. *Bioconj Chem* 2019;30:1067–76.  
667 <https://doi.org/10.1021/acs.bioconjchem.9b00015>.

668 [52] Wan T, Lu S, Cheng W, Ren J, Wang M, Hu B, et al. A spectroscopic and  
669 theoretical investigation of interaction mechanisms of tetracycline and polystyrene  
670 nanospheres under different conditions. *Environ Pollut* 2019;398–405.  
671 <https://doi.org/10.1016/j.envpol.2019.03.049>.

672 [53] Pessoni L, Veclin C, Hadri HE, Cugnet C, Davranche M, Pierson-Wickmann A-  
673 C, et al. Soap- and metal-free polystyrene latex particles as a nanoplastic model. *Environ*  
674 *Sci Nano* 2019;6:2253–8. <https://doi.org/10.1039/C9EN00384C>.

675 [54] Al-Sid-Cheikh M, Rowland SJ, Stevenson K, Rouleau C, Henry TB, Thompson  
676 RC. Uptake, Whole-Body Distribution, and Depuration of Nanoplastics by the Scallop  
677 *Pecten maximus* at Environmentally Realistic Concentrations. *Environ Sci Technol* 2018.  
678 <https://doi.org/10.1021/acs.est.8b05266>.

679 [55] Mitrano DM, Beltzung A, Frehland S, Schmiedgruber M, Cingolani A, Schmidt  
680 F. Synthesis of metal-doped nanoplastics and their utility to investigate fate and  
681 behaviour in complex environmental systems. *Nat Nanotechnol* 2019;14:362–8.  
682 <https://doi.org/10.1038/s41565-018-0360-3>.

683 [56] Jiang X, Tian L, Ma Y, Ji R. Quantifying the bioaccumulation of nanoplastics and  
684 PAHs in the clamworm *Perinereis aibuhitensis*. *Sci Total Environ* 2019;655:591–7.  
685 <https://doi.org/10.1016/j.scitotenv.2018.11.227>.

686 [57] Catarino AI, Frutos A, Henry TB. Use of fluorescent-labelled nanoplastics (NPs)  
687 to demonstrate NP absorption is inconclusive without adequate controls. *Sci Total*  
688 *Environ* 2019;670:915–20. <https://doi.org/10.1016/j.scitotenv.2019.03.194>.

689 [58] Magrì D, Sánchez-Moreno P, Caputo G, Gatto F, Veronesi M, Bardi G, et al.  
690 Laser Ablation as a Versatile Tool To Mimic Polyethylene Terephthalate Nanoplastic  
691 Pollutants: Characterization and Toxicology Assessment. *ACS Nano* 2018;12:7690–700.  
692 <https://doi.org/10.1021/acsnano.8b01331>.

693 [59] González-Pleiter M, Tamayo-Belda M, Pulido-Reyes G, Amariei G, Leganés F,  
694 Rosal R, et al. Secondary nanoplastics released from a biodegradable microplastic  
695 severely impact freshwater environments. *Environ Sci Nano* 2019.

696 [60] El Hadri H, Gigault J, Maxit B, Grassl B, Reynaud S. Nanoplastic from  
697 mechanically degraded primary and secondary microplastics for environmental  
698 assessments. *NanoImpact* 2020;17:100206.  
699 <https://doi.org/10.1016/j.impact.2019.100206>.

700 [61] Rodríguez-Hernández AG, Muñoz-Tavares JA, Aguilar-Guzmán C, Vazquez-  
701 Duhalt R. Novel and simple method for polyethylene terephthalate (PET) nanoparticles  
702 production. *Environ Sci Nano* 2019. <https://doi.org/10.1039/C9EN00365G>.

703 [62] Tian L, Chen Q, Jiang W, Wang L, Xie H, Kalogerakis N, et al. A carbon-14  
704 radiotracer-based study on the phototransformation of polystyrene nanoplastics in water:

705 Versus in air. *Environ Sci Nano* 2019;6:2907–17. <https://doi.org/10.1039/c9en00662a>.

706 [63] Astner AF, Hayes DG, O’Neill H, Evans BR, Pingali SV, Urban VS, et al.

707 Mechanical formation of micro- and nano-plastic materials for environmental studies in

708 agricultural ecosystems. *Sci Total Environ* 2019;685:1097–106.

709 <https://doi.org/10.1016/j.scitotenv.2019.06.241>.

710 [64] Enfrin M, Lee J, Gibert Y, Basheer F, Kong L, Dumée LF. Release of hazardous

711 nanoplastic contaminants due to microplastics fragmentation under shear stress forces. *J*

712 *Hazard Mater* 2020;384:121393. <https://doi.org/10.1016/j.jhazmat.2019.121393>.

713 [65] Wang X, Bolan N, Tsang DCW, Sarkar B, Bradney L, Li Y. A review of

714 microplastics aggregation in aquatic environment: Influence factors, analytical methods,

715 and environmental implications. *J Hazard Mater* 2021;402.

716 <https://doi.org/10.1016/j.jhazmat.2020.123496>.

717 [66] Hotze EM, Phenrat T, Lowry GV. Nanoparticle Aggregation: Challenges to

718 Understanding Transport and Reactivity in the Environment. *J Environ Qual* 2010;39:16.

719 [67] Holthoff H, Egelhaaf SU, Borkovec M, Schurtenberger P, Sticher H. Coagulation

720 Rate Measurements of Colloidal Particles by Simultaneous Static and Dynamic Light

721 Scattering. *Langmuir* 1996;12:5541–9. <https://doi.org/10.1021/la960326e>.

722 [68] Oncsik T, Trefalt G, Csendes Z, Szilagy I, Borkovec M. Aggregation of

723 Negatively Charged Colloidal Particles in the Presence of Multivalent Cations. *Langmuir*

724 2014;30:733–41. <https://doi.org/10.1021/la4046644>.

725 [69] Veclin Cloé et al. Effect of the surface hydrophobicity-morphology-functionality

726 of nanoplastics on their homo-aggregation in seawater. *ACS EST Water* n.d.:accepted.

727 [70] Singh N, Tiwari E, Khandelwal N, Darbha GK. Understanding the stability of

728 nanoplastics in aqueous environments: Effect of ionic strength, temperature, dissolved

729 organic matter, clay, and heavy metals. *Environ Sci Nano* 2019;6:2968–76.

730 <https://doi.org/10.1039/c9en00557a>.

731 [71] Liu Y, Hu Y, Yang C, Chen C, Huang W, Dang Z. Aggregation kinetics of UV

732 irradiated nanoplastics in aquatic environments. *Water Res* 2019;163.

733 <https://doi.org/10.1016/j.watres.2019.114870>.

734 [72] Liu Y, Huang Z, Zhou J, Tang J, Yang C, Chen C, et al. Influence of

735 environmental and biological macromolecules on aggregation kinetics of nanoplastics in

736 aquatic systems. *Water Res* 2020;186. <https://doi.org/10.1016/j.watres.2020.116316>.

737 [73] Mao Y, Li H, Huangfu X, Liu Y, He Q. Nanoplastics display strong stability in

738 aqueous environments: Insights from aggregation behaviour and theoretical calculations.

739 *Environ Pollut* 2020;258:113760. <https://doi.org/10.1016/j.envpol.2019.113760>.

740 [74] Singh N, Bhagat J, Tiwari E, Khandelwal N, Darbha GK, Shyama SK. Metal

741 oxide nanoparticles and polycyclic aromatic hydrocarbons alter nanoplastic’s stability

742 and toxicity to zebrafish. *J Hazard Mater* 2020.

743 <https://doi.org/10.1016/j.jhazmat.2020.124382>.

744 [75] Sun H, Jiao R, Wang D. The difference of aggregation mechanism between

745 microplastics and nanoplastics: Role of Brownian motion and structural layer force.

746 *Environ Pollut* 2020;115942. <https://doi.org/10.1016/j.envpol.2020.115942>.

747 [76] Yu S, Shen M, Li S, Fu Y, Zhang D, Liu H, et al. Aggregation kinetics of

748 different surface-modified polystyrene nanoparticles in monovalent and divalent

749 electrolytes. *Environ Pollut* 2019;255:113302.

750 <https://doi.org/10.1016/j.envpol.2019.113302>.

751 [77] Li X, He E, Xia B, Van Gestel CAM, Peijnenburg WJGM, Cao X, et al. Impact of  
752 CeO<sub>2</sub> nanoparticles on the aggregation kinetics and stability of polystyrene nanoplastics:  
753 Importance of surface functionalization and solution chemistry. *Water Res* 2020;186.  
754 <https://doi.org/10.1016/j.watres.2020.116324>.

755 [78] Nolte TM, Hartmann NB, Kleijn JM, Garnæs J, van de Meent D, Jan Hendriks A,  
756 et al. The toxicity of plastic nanoparticles to green algae as influenced by surface  
757 modification, medium hardness and cellular adsorption. *Aquat Toxicol* 2017;183:11–20.  
758 <https://doi.org/10.1016/j.aquatox.2016.12.005>.

759 [79] Shams M, Alam I, Chowdhury I. Aggregation and stability of nanoscale plastics  
760 in aquatic environment. *Water Res* 2020;171:115401.  
761 <https://doi.org/10.1016/j.watres.2019.115401>.

762 [80] Wang J, Zhao X, Wu A, Tang Z, Niu L, Wu F, et al. Aggregation and stability of  
763 sulfate-modified polystyrene nanoplastics in synthetic and natural waters. *Environ Pollut*  
764 2021;268. <https://doi.org/10.1016/j.envpol.2020.114240>.

765 [81] Pradel A, Ferreres S, Veclin C, El Hadri H, Gautier M, Grassl B, et al.  
766 Stabilization of Fragmental Polystyrene Nanoplastic by Natural Organic Matter: Insight  
767 into Mechanisms. *ACS EST Water* 2021:acsestwater.0c00283.  
768 <https://doi.org/10.1021/acsestwater.0c00283>.

769 [82] Dong S, Cai W, Xia J, Sheng L, Wang W, Liu H. Aggregation kinetics of  
770 fragmental PET nanoplastics in aqueous environment: Complex roles of electrolytes, pH  
771 and humic acid. *Environ Pollut* 2021;268:115828.  
772 <https://doi.org/10.1016/j.envpol.2020.115828>.

773 [83] Cai L, Hu L, Shi H, Ye J, Zhang Y, Kim H. Effects of inorganic ions and natural  
774 organic matter on the aggregation of nanoplastics. *Chemosphere* 2018;197:142–51.  
775 <https://doi.org/10.1016/j.chemosphere.2018.01.052>.

776 [84] Oriekhova O, Stoll S. Heteroaggregation of nanoplastic particles in the presence  
777 of inorganic colloids and natural organic matter. *Environ Sci Nano* 2018;5:792–9.  
778 <https://doi.org/10.1039/c7en01119a>.

779 [85] Wu J, Jiang R, Lin W, Ouyang G. Effect of salinity and humic acid on the  
780 aggregation and toxicity of polystyrene nanoplastics with different functional groups and  
781 charges. *Environ Pollut* 2019;836–43. <https://doi.org/10.1016/j.envpol.2018.11.055>.

782 [86] Tallec K, Blard O, González-Fernández C, Brotons G, Berchel M, Soudant P, et  
783 al. Surface functionalization determines behavior of nanoplastic solutions in model  
784 aquatic environments. *Chemosphere* 2019;225:639–46.  
785 <https://doi.org/10.1016/j.chemosphere.2019.03.077>.

786 [87] Zhang Y, Luo Y, Guo X, Xia T, Wang T, Jia H, et al. Charge mediated interaction  
787 of polystyrene nanoplastic (PSNP) with minerals in aqueous phase. *Water Res* 2020;178.  
788 <https://doi.org/10.1016/j.watres.2020.115861>.

789 [88] Feng L-J, Wang J-J, Liu S-C, Sun X-D, Yuan X-Z, Wang S-G. Role of  
790 extracellular polymeric substances in the acute inhibition of activated sludge by  
791 polystyrene nanoparticles. *Environ Pollut* 2018;238:859–65.  
792 <https://doi.org/10.1016/j.envpol.2018.03.101>.

793 [89] Grassi G, Gabellieri E, Cioni P, Paccagnini E, Faleri C, Lupetti P, et al. Interplay  
794 between extracellular polymeric substances (EPS) from a marine diatom and model  
795 nanoplastic through eco-corona formation. *Sci Total Environ* 2020;725.  
796 <https://doi.org/10.1016/j.scitotenv.2020.138457>.

797 [90] Besseling E, Quik JTK, Sun M, Koelmans AA. Fate of nano- and microplastic in  
798 freshwater systems: A modeling study. *Env Pollut* 2017;220:540–8.

799 [91] Venel Z, Tabuteau H, Pradel A, Pascal PY, Grassl B, El Hadri H, et al.  
800 Environmental Fate Modeling of Nanoplastics in a Salinity Gradient Using a Lab-on-a-  
801 Chip: Where Does the Nanoscale Fraction of Plastic Debris Accumulate? *Environ Sci*  
802 *Technol* 2021. <https://doi.org/10.1021/acs.est.0c07545>.

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#### 804 **Conflicts of Interest**

805 There are no conflicts of interest to declare.

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