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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 μm behaved so differently from larger
74 microplastics (1 μm 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 μm , lower mesh size down to 64 μm 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 μm), 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.

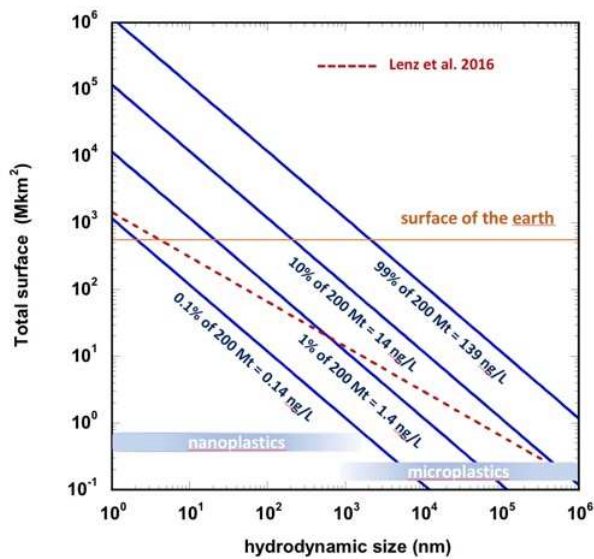
214

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 (Ca^{2+}) destabilize nanoplastics at lower concentrations than
357 monovalent cations (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⁻¹), hydrophobicity
374 (surface energy, 2.20 to 37.5 mJm⁻²), 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⁻¹) than anisotropic nanoplastics (CCC ~ 100 mmol L⁻¹) 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, SO₃H, C₂H₂O) 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 ₂						
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 ₃ H	90	159	12	6	DLS	commercial	[80]		
	80	264	29	5	[77]				
PSL-C ₂ H ₂ 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 ₂ O ₂ – 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]		

401

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 CaCl₂, NaCl, and FeCl₂ 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-NH₂
409 in the presence of Fe₂O₃ and alginate as model organic matter. Due to the amidine function,
410 the ratio of the concentration of natural organic matter *over* PSL-NH₂ 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-NH₂ 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-NH₂ 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 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 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., CeO_2 -nanoparticle of 25 nm) and humic
446 acid was influenced by the nanoplastic surface chemical functionality (COOH , SO_3H ,
447 $\text{C}_2\text{H}_2\text{O}$, NH_2), the pH, and the presence of a divalent electrolyte (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.

492

493

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**

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510 as:

511 * of special interest

512 ** of outstanding interest+

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804 **Conflicts of Interest**

805 There are no conflicts of interest to declare.

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