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pH-Dependent Silica Nanoparticle Dissolution and 1 Cargo Release

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1 **pH-Dependent Silica Nanoparticle Dissolution and**
2 **Cargo Release**

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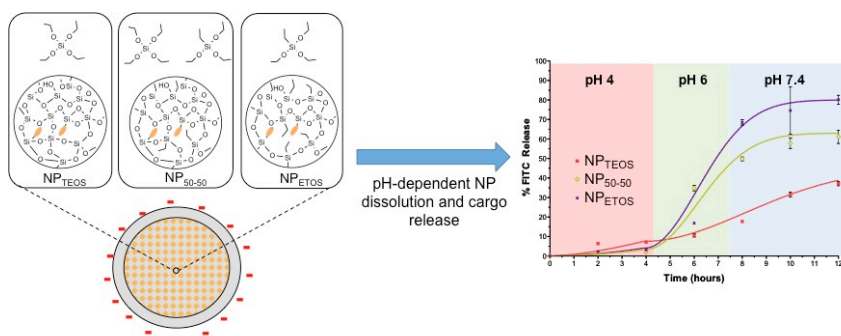
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30 **Abstract**

31 The dissolution of microporous silica nanoparticles (NP) in aqueous environments of different
32 biologically relevant pH was studied in order to assess their potential as drug delivery vehicles.
33 Silica NPs, loaded with fluorescein, were prepared using different organosilane precursors
34 (tetraethoxysilane, ethyl triethoxysilane or a 1:1 molar ratio of both) and NP dissolution was
35 evaluated in aqueous conditions at pH 4, pH 6 and pH 7.4. These conditions correspond to
36 the acidity of the intracellular environment (late endosome, early endosome, cytosol
37 respectively) and gastrointestinal tract ('fed' stomach, duodenum and jejunum respectively).
38 All NPs degraded at pH 6 and pH 7.4, while no dissolution was observed at pH 4. NP dissolution
39 could be clearly visualised as mesoporous hollows and surface defects using electron
40 microscopy, and was supported by UV-Vis, fluorimetry and DLS data. The dissolution profiles
41 of the NPs are particularly suited to the requirements of oral drug delivery, whereby NPs must
42 resist degradation in the harsh acidic conditions of the stomach (pH 4), but dissolve and
43 release their cargo in the small intestine (pH 6 - 7.4). Particle cores made solely of ethyl
44 triethoxysilane exhibited a 'burst release' of encapsulated fluorescein at pH 6 and pH 7.4,
45 whereas NPs synthesised with tetraethoxysilane released fluorescein in a more sustained
46 fashion. Thus, by varying the organosilane precursor used in NP formation, it is possible to
47 modify particle dissolution rates and tune the release profile of encapsulated fluorescein. The
48 flexible synthesis afforded by silica NPs to achieve pH-responsive dissolution therefore makes
49 this class of nanomaterial an adaptable platform that may be well suited to oral delivery
50 applications.

51 **Graphical Abstract**

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54



55 **Introduction**

56

57 Nanoparticle (NP)-based delivery systems have come to prominence over the past two
58 decades as they can be designed to carry poorly soluble drugs or molecules that are
59 prone to degradation in biological conditions.¹⁻⁴ NPs can also transport therapeutics
60 across highly regulated biological boundaries such as the blood brain barrier.^{5,6} In
61 particular, silica NPs (SiNPs) are regularly described as excellent candidates for drug
62 delivery applications because they are regarded as biocompatible⁷⁻⁹ and inert.¹⁰
63 However, it is the adaptable and flexible nature of siloxane chemistry that makes this
64 class of nanomaterial so widely studied as a drug delivery agent. This is facilitated, in
65 part, by the large number of commercially available organosiloxane derivatives that
66 can be used as precursors for SiNP synthesis. The chemistries of these precursors can
67 vary widely and means that SiNPs can exhibit a range of useful physicochemical
68 properties (e.g. different porosity, charge, hydrophobicity), which, in turn, allows for
69 different kinds of therapeutics to be encapsulated and delivered to disease sites.

70 Most silica-based drug delivery studies employ mesoporous silica, having pore
71 sizes of the order 2-50nm, and rely on tunable cargo release via a 'gatekeeper'
72 strategy.^{8,11-14} Despite their popularity, the requirement to load cargo and incorporate
73 gatekeepers after NP synthesis introduces additional complexity to particle design. On
74 the other hand, microporous silica NPs have characteristic pores of less than 2nm¹⁵,
75 that are challenging to characterise accurately with appropriate methods and
76 expertise compared to mesoporous silica.¹⁶ Encapsulatation of different therapeutics
77 can be achieved during NP synthesis^{2,17,18} and the release mechanism is via the natural
78 degradation of the silica.¹⁹ The process of NP degradation is therefore largely governed
79 by the organosiloxane precursors, and their associated physicochemical properties,
80 that can be easily imparted during synthesis. However, microporous silica remains
81 understudied as a drug delivery candidate and is more frequently reported in
82 immunoassays²⁰⁻²² and bioimaging.^{9,23-25} This is surprising, considering the adaptable
83 nature of silica and the fact that it, in comparison to its mesoporous counterpart,
84 avoids the need for gatekeeping to control drug release and the associated
85 complications related to cargo leeching. We therefore feel microporous silica NPs are

86 an interesting nanomaterial to study and have the potential to impact the drug delivery

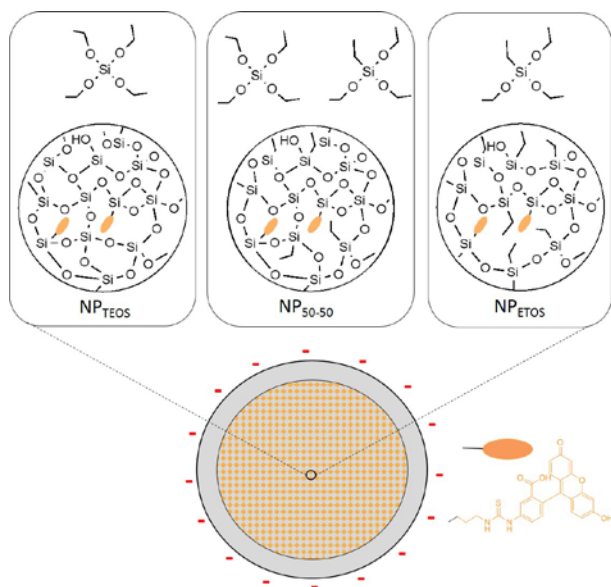


Figure 1: Silica NPs were prepared with different core chemistries by employing different NP precursors during synthesis: tetraethoxysilane (TEOS) or ethyl triethoxysilane (ETOS). These NPs were called NP_{TEOS} and NP_{ETOS}. TEOS and ETOS were also added in an equal molar ratio (NP₅₀₋₅₀). Covalently binding fluorescein (FITC) in the NP cores also provided information about particle degradation and cargo release.

87 field.

88 We hypothesise the development of a dissolution-based method of controllably
89 releasing encapsulated cargo from microporous SiNPs by synthesising colloids using
90 different organosiloxane precursors. SiNPs are formed utilising hydrolysis but this pH-
91 dependent mechanism is reversible and suggests SiNPs may degrade at different rates
92 in different acidic conditions.

93 Intracellular NP-drug delivery typically requires endocytosis of the nanocarrier
94 to transport a therapeutic across the cell membrane. Trafficking of the NPs from the
95 extracellular environment (pH 7.4) into early endosomes (pH 6) and then to late
96 endosomes/lysosomes (pH 4) means environments of different acidity are
97 experienced. The same can be said for oral drug delivery applications in which
98 medicines first encounter the harsh environment of the stomach (pH 4 in 'fed state')
99 and are then passed to the duodenum (pH 6) and jejunum (pH 7.4) for adsorption.

100 We have synthesised core-shell SiNPs via the reverse microemulsion method
101 (Figure 1) and investigated their dissolution in aqueous conditions at biologically
102 relevant pH (pH 4, pH 6, pH 7.4), similarly to other NP dissolution studies.²⁶⁻²⁹ Different
103 siloxane precursors were employed during the core formation in order to produce

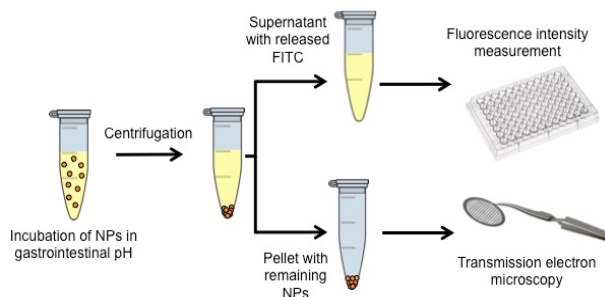


Figure 2: NPs were synthesised using tetraethoxysilane (NP_{TEOS}), ethyl triethoxysilane (NP_{ETOS}) or equal ratio of both (NP₅₀₋₅₀), and were degraded in biologically relevant pH. Dissolution of the NPs was assessed by fluorimetry (FITC release from the NPs) and electron microscopy (NP morphology and integrity).

104 particles that exhibit varying degrees of hydrophobicity, which in turn may be able to
105 affect NP dissolution and the ability to host different cargos. A shell composed of
106 tetraethoxysilane (TEOS) and negatively charged phosphonates was then added to
107 each set of particles to insure similar surface chemistry.

108 The precursors used for core formation were TEOS, ethyl triethoxysilane (ETOS),
109 bis(triethoxysilyl)benzene and bis(triethoxysilyl)biphenyl. However, the colloids formed
110 using the aromatic oxysilanes were unstable in aqueous conditions and only particles
111 formed using TEOS and ETOS were studied to assess dissolution. Degradation and
112 release of the encapsulated cargo (i.e. fluorescein; FITC) from the SiNPs were
113 monitored by electron microscopy and fluorimetry (Figure 2), and stability studies
114 were carried out using dynamic light scattering (DLS). Overall, negligible dissolution
115 was observed at pH 4 and suggested the NPs may survive the acidic conditions of the
116 stomach or cellular lysosome, thus minimising cargo release. NP degradation was
117 accelerated in pH 6 and pH 7.4 and may support the release the encapsulated cargo in
118 small intestinal pH, at physiological pH or in early endosomes. A study mimicking
119 progress through the GI tract (i.e. pH 4 to pH6 to pH 7.4) then showed the NPs released
120 fluorescein in a pH-dependent manner, with NPs formed using more ETOS exhibiting
121 'burst' release profiles and those formed solely using TEOS displaying 'slow' release.

122 **Methods**

123 NPs synthesis and characterisation: materials, procedures, size and ζ -potential
124 analysis, TEM studying of NP dissolution are detailed in the Supporting Information

125 FITC-release assay: The degree of FITC release was evaluated by measuring the amount
126 of dye present in the supernatant and comparing the values measured with the
127 fluorescent-based calibration curve for FITC at the corresponding pH. The values
128 achieved from the independent experiments are reported as average ($n = 3$) \pm SD. A
129 Tecan Infinite M200 Pro Safire microplate reader was used for absorbance and
130 fluorescence emission measurements. Samples were added to Nunc Maxisorb 96 well
131 plates before being read (490/525 nm, $\lambda_{ex}/\lambda_{em}$). 250 μ g of NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS}
132 were washed once by centrifugation and re-dispersion in water before dispersion in 1
133 ml of each phosphate buffer (pH 4, 6 or 7.4). For each sample in each buffer, 7 samples
134 were prepared, one for each timepoint (1, 2, 4, 6, 8, 10, 24hrs) and shaken at 37°C (600
135 rpm). After each incubation time, samples were centrifuged (14000rpm, 10 min) and
136 700 μ L of supernatant were removed and the remainder discarded. The pellet isolated
137 after centrifugation was washed twice by centrifugation and re-dispersion in water,
138 then used for TEM analysis.

139 GI tract-like assay: 200 μ g of NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS} were washed once by
140 centrifugation and re-dispersion in water before dispersion in 1 mL of phosphate
141 buffer at pH 4. The samples were shaken at 37°C (600 rpm). After 2 hours the samples
142 were centrifuged, 300 μ L of the supernatant was measured ($\lambda_{ex}/\lambda_{em}$, 490/525 nm,
143 100 μ L per well). The remaining NP suspensions were filled with 300 μ L of fresh buffer
144 pH 4 and re-incubated. After 2hr the samples were centrifuged and the supernatants
145 completely removed and used for the fluorescence analysis, while the pellets were re-
146 dispersed in 1 mL of buffer at pH 6 and shaken at 37°C (600 rpm). After 2 hours, the
147 samples were centrifuged and the supernatants completely removed and used for the
148 fluorescence analysis, while the pellets were re-dispersed in 1mL of buffer pH 7.4 and
149 shaken again. The samples were centrifuged every 2 hours, 300 μ L of the supernatant
150 were used to fill three wells of a 96-well plate and the fluorescence was measured. The
151 experiment was stopped after 12hrs.

152

153 **Results and Discussion**

154 Core-shell microporous SiNPs were synthesised via the reverse microemulsion
 155 method^{30,31} and their dissolution in biologically relevant pH was investigated. Different
 156 organosiloxane precursors were employed during core formation to produce particles
 157 with varying degrees of hydrophobicity and core crosslinking densities. FITC was
 158 modified with aminopropyl trimethoxysilane via thiourea bond formation and enabled
 159 the dye to be covalently incorporated into the silica matrix during core formation
 160 alongside the SiNP precursors (Figure 1).^{31,32} A shell composed of TEOS and negatively
 161 charged phosphonates was then added to each set of particles to insure similar surface
 162 chemistry.³³ From the organosiloxane analogues chosen for this study,
 163 tetraethoxysilane (TEOS), the traditional SiNP precursor, and ethyl triethoxysilane
 164 (ETOS) were the only analogues capable of forming colloids that were stable in
 165 aqueous conditions. These NPs have been named NP_{TEOS} and NP_{ETOS} respectively. TEOS
 166 and ETOS were also added to the microemulsion in equal molar ratios, thus yielding a
 167 third batch of NPs: NP₅₀₋₅₀.

168 Two other siloxanes, bis(triethoxysilyl)benzene and bis(triethoxysilyl)biphenyl,
 169 were also used alongside TEOS as precursors for NP core formation. It was possible to
 170 generate stable NPs in ethanol using both siloxanes but they visually aggregated in less
 171 than one minute when transferred to DI water (Figure S1). Their rapid aggregation was
 172 attributed to the hydrophobic nature of their aromatic moiety and their potential to π -
 173 stack in water, and suggests further surface chemical modification (such as by
 174 PEGylation) would be needed to increase solubility in biological conditions. Even NPs
 175 formed using a 95:5 TEOS:bis(triethoxysilyl)benzene visually aggregated in aqueous
 176 medium (Figure S2).

177
 178 *Table 1: Physicochemical characterisation of the NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS} by DLS and TEM. FITC loading per NP*
 179 *was also quantified and allowed for percentage of FITC release to be determined in later dissolution experiments*
 180 *(n=3).*

	DLS			TEM	Loading
	Z-Av. \emptyset (nm)	PDI	ζ -potential (mV)	\emptyset (nm)	FITC per NP
NP_{TEOS}	132.5 \pm 1.3	0.177 \pm 0.016	-27.8 \pm 0.80	72 \pm 8	1256 \pm 389
NP₅₀₋₅₀	170.0 \pm 2.2	0.147 \pm 0.005	-24.0 \pm 0.27	80 \pm 13	1578 \pm 574
NP_{ETOS}	222.9 \pm 6.0	0.275 \pm 0.030	-22.3 \pm 0.65	50 \pm 31	122 \pm 27

181

182 The three NPs (NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS}) were characterised by DLS and
183 transmission electron microscopy (TEM) in order to quantify particle size and surface
184 charge (Table 1). Using TEM, the NP diameters were measured to be 72±8 nm, 80±13
185 nm and 50±31 nm for NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS} respectively. However, using DLS, the
186 size (Z-average) of the NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS} was 132.5±1.3 nm, 170.0±2.2 nm,
187 222.9±6.0 nm. The NP Z-average size increased with the increasing proportion of ETOS,
188 which was accompanied by the decrease of the absolute values of overall negative
189 charge for the three NPs: -27.8±0.80 mV, -24.0±0.27 mV, -22.3±0.65 mV for NP_{TEOS},
190 NP₅₀₋₅₀ and NP_{ETOS}. This inverted correlation suggested that the NPs became less
191 colloidal stable and experienced some degree of aggregation when more the
192 hydrophobic ETOS was used during NP synthesis. No dramatic aggregation over a
193 period of 2 days was observed for the NP_{TEOS} and NP₅₀₋₅₀ at pH 4, pH 6 and pH 7.4
194 buffers, but at pH 4, the NP_{ETOS} diameter increased gradually to 1µm (Figure 3). This
195 effect is not desirable for drug delivery systems as increased NP size reduces the overall
196 surface area-to-volume ratio, which is detrimental to controlled drug release,
197 significantly changes the size-dependent properties of the NPs and may affect NP-cell
198 interactions. However, in the case of *in vivo* drug delivery this is unlikely to be
199 problematic since, in the case of oral administration, the residence time of food in the
200 stomach is typically 4 hours or less. For intracellular delivery, NPs are likely to be firstly
201 administered intravenously before reaching a tumour site (i.e. at pH 7.4 where they
202 are stable). NP localisation in organs usually only then takes a matter of hours, during
203 which time they are endocytosed and eventually trafficked to late endosomes/
204 lysosomes (pH 4).

205 The dissolution of SiNPs is well described in the literature and is caused by
206 hydrolysis of the silica matrix, which is accelerated at higher pH and temperature.^{21,34}
207 Park et al described the hollowing of SiNPs due to etching under basic conditions.³⁵

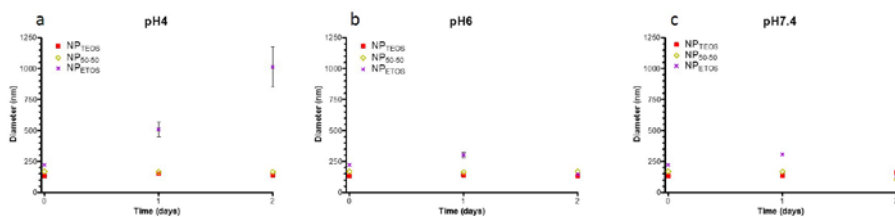
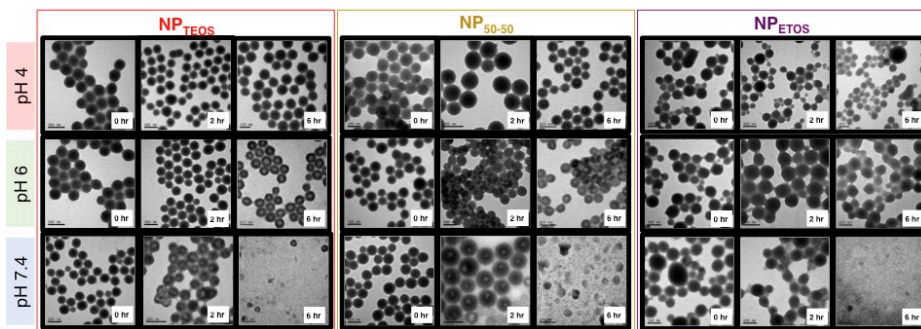


Figure 3: DLS analysis of NP size (Z-average, $n=3$) over a 2 day period in the pH 4, pH 6 and pH 7.4 buffers. (a) NP_{TEOS} and NP_{50-50} were stable over time, but NP_{ETOS} gradually aggregated into micron-sized particles over 48 hours. (b) All NPs remained colloidally stable for 2 days at pH 6. (c) At pH7.4, the three sets of NPs also retained their colloidal stability for 2 days.

208 The authors suggested that small ‘seed pores’ in the particle matrix merge to form
209 single voids and eventually results in large hollows. Mahon et al. demonstrated that
210 SiNPs can degrade during *in vitro* cellular experimentation and observed NP hollowing
211 by TEM following particle incubation in cell culture medium at 37°C.³⁴ We have also
212 recently observed hollowing in a ‘dissolution assay’ designed to exploit SiNP
213 degradation as a way to improve immunoassay signal-to-noise ratios.²¹

214 To this end, we have incubated NP_{TEOS} , NP_{50-50} and NP_{ETOS} in buffered solutions
215 at pH 4, pH 6 and pH 7.4 and analysed the NP integrity (i.e. the presence/absence of
216 cavities/hollows) as an indicator of degradation. Clear changes in NPs morphology
217 were observed after 6 hours at 37°C (Figure 4), and a complete 24 hour degradation
218 study by TEM is presented in the Supporting Information (Figure S3, S4, S5). It is
219 evident from Figure 4 that no changes in particle morphology were found for NP_{TEOS} ,
220 NP_{50-50} or NP_{ETOS} when incubated at pH4. Small mesopore-sized hollows only became
221 visible at pH4 in NP_{TEOS} after 24 hours of incubation (Figure S3, Table S3). This suggests
222 that the three types of SiNPs would be robust enough to remain intact in the stomach
223 (‘fed state’) and presumably also in the ‘fasted state’ (approx. pH 1.2)^{36,37} because
224 particle hydrolysis would be slower in more acidic conditions. However, the NPs would
225 not be capable of intracellular dissolution-based cargo release if the colloids were
226 eventually trafficked to lysosomes.

227 At pH 6, we noticed that degradation of the colloids had occurred in the NP_{TEOS}
 228 and NP₅₀₋₅₀, but was not evident in the NP_{ETOS} particles. For the NP₅₀₋₅₀ samples, clear
 229 mesopore-scale hollows measuring 13.71 ± 4.93 nm in diameter in the could be seen



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Figure 4: TEM of NP_{TEOS}, NP₅₀₋₅₀, and NP_{ETOS} incubated over time in pH 4, pH 6 and pH 7.4 solutions. No changes in NP morphology were observed in pH 4 over time which suggested silica NPs may be capable of enduring the harsh conditions of the stomach. NPs dissolved in pH 6 and pH7.4 due to the increased rate of hydrolysis of the silica matrix. Differences were observed in the mode of dissolution of NP_{TEOS} and NP₅₀₋₅₀ compared to NP_{ETOS}: Hollowing of the particle core was present in NP_{TEOS} and NP₅₀₋₅₀, whereas NP_{ETOS} degradation appeared to begin at the particle exterior surface.

230 after 2 hours and was further evidenced by the micrographs taken from 6 to 24 hours
 231 in which the etching is seen to be further enhanced (Fig S4, Table 3). NP_{TEOS} did not
 232 exhibit visible degradation at 2 hours at pH 6 but 6.96 ± 3.73 nm hollows were clearly
 233 evident after 6 hours. Such hollowed structures are consistent with those found in
 234 other studies focussed on SiNP degradation.^{21,34,35} Interestingly NP_{ETOS} exhibited no
 235 visual hollowing in the NP core at pH 6, which is presumably a result of the hydrophobic
 236 ethyl groups reducing the presence of water in the silica matrix, thus inhibiting the
 237 hydrolysis of the -O-Si-O- bond. Interestingly, it appeared that NP_{ETOS} underwent a
 238 dissolution process that led to gradual disintegration of the exterior particle surface.
 239 The apparent method of NP_{ETOS} degradation is therefore different to that of NP_{TEOS} and
 240 NP₅₀₋₅₀, and is presumably linked to the hydrophobic/hydrophilic nature of the
 241 respective particle cores. It is possible that the more hydrophilic cores of NP_{TEOS} and
 242 NP₅₀₋₅₀ are susceptible to initial etching by hydrolysis and followed the 'seed pore'
 243 phenomenon³⁵ to eventually form mesoscopic cavities. On the other hand, the
 244 hydrophobic NP_{ETOS} core resisted hydrolysis and dissolution occurred at the particle
 245 exterior that was formed only by using TEOS.

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246 A striking difference in NP integrity was found for particles incubated in pH 7.4
247 buffer. NP_{TEOS} and NP₅₀₋₅₀ exhibited more severe etching after 2 hours incubation
248 compared to pH 6, which is in agreement with the hypothesis that increased basic
249 conditions lead to more rapid silica hydrolysis and particle dissolution. Indeed, it is
250 clear from the TEM images that NP_{TEOS} and NP₅₀₋₅₀ exhibited an evolution from a
251 microporous structure to a hollowed mesoporous one, which can increase the overall
252 NP surface area and further enhance degradation. This accelerated NP dissolution for
253 both sets of NPs at pH 7.4 caused NP_{TEOS} and NP₅₀₋₅₀ to be largely degraded after 6
254 hours. TEM showed very few intact particles and features observed were
255 predominantly NP debris, which agrees with previous SiNP degradation studies.²¹
256 Further analysis of the NP hollows was conducted by scanning transmission electron
257 microscopy (Figure S6). The results show that the hollowed interior the NPs could
258 eventually etch through to the surface of NP_{TEOS} and NP₅₀₋₅₀ as a way of reducing
259 surface energy,³⁵ and resulted in distinct surface deformations of the NPs.

260 The fact that NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS} all degraded at pH7.4 is promising for
261 oral drug delivery as the jejunum (pH 7.4) exhibits larger villi compared to do
262 duodenum (pH 6). This means drugs released at this point in the GI tract would be
263 readily absorbed, thus improving bioavailability before proceeding to enterohepatic
264 circulation. Considering that the NP matrix almost completely disintegrates under
265 these conditions, it may avoid any potential nanotoxicity issues and be cleared from
266 the body. Indeed, silica is used in the food industry as a bulking agent in a number of
267 food products (E551; silicium dioxide) and has been reported to degrade into
268 biocompatible silicic acid.³⁸ However, dissolution of the NPs at pH 7.4 poses a challenge
269 for intracellular delivery as this strategy first involves intravenous NP injection, which
270 exposes the NPs to a pH 7.4 environment, and suggests some of the encapsulated
271 cargo would diffuse from the nanomaterial before localisation. In turn, the total
272 amount of drug transported across the cell membrane would be reduced.

273 Fluorescein isothiocyanate (FITC) was covalently bound inside the core of
274 NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS} and served as an indicator of NP degradation. The release
275 profile of FITC into solution can therefore be used to infer the extent of NP dissolution
276 and can be corroborated with the TEM images. Due to the hydrophobic nature of FITC
277 (an analogue for poorly soluble drugs), monitoring the dye release also allowed for

278 concurrent assessment of the release profile of small molecules from the three sets of
279 NPs over time. At each time point, the NPs were centrifuged and intact NPs were
280 concentrated into a pellet, thus allowing the supernatant to be used for analysing free
281 FITC released from the NPs (Figure 2). The quantity of dye released was then
282 extrapolated from the calibration curves of known FITC concentrations prepared at the
283 three different pHs in order to account for FITC's pH-dependent fluorescence emission
284 intensity. The results of this FITC release study are presented in Figure 5.

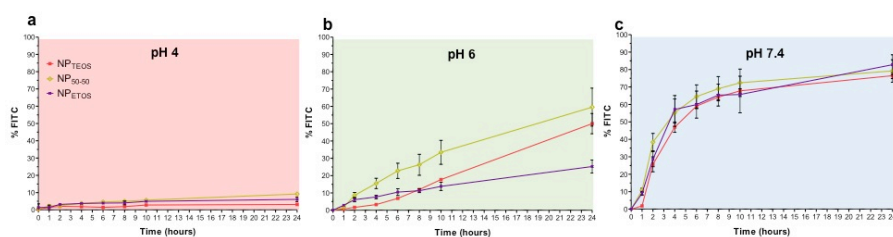


Figure 5: FITC release from the NPs over time when incubated in gastrointestinal pH's. (a) NPs were incubated in pH 4 solution and little FITC was detected in the sample supernatant over a 24 hour period. (b) In pH 6 gradual FITC release was observed and was attributed to increased hydrolysis rate compared to pH 4. (c) NP degradation was most rapid in pH 7.4 and release the majority of the FITC cargo into solution over time.

285 FITC release from NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS} was minimal at pH 4 over the course
286 of 24 hours (Figure 5a). The overall concentration of released FITC was less than 5%
287 after 2 hours in the acidic environment and when considering the images of intact NPs
288 obtained via TEM (Figure 4), it suggests that the SiNPs employed in this study would
289 be capable of resisting degradation in the stomach. They may therefore be able to
290 reliably carry drugs to the intestine, and agrees with other reports focussed on silica
291 NP integrity in the stomach and the GI tract as a whole.^{36,37} This was further supported
292 by the fact that less than 10% of FITC was released from NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS} at
293 pH 4 after 24 hours. In addition, the low release rate of the dye into solution suggested
294 SiNPs intracellularly trafficked to late endosomes/lysosomes would not release
295 encapsulated cargo via NP dissolution and alternative strategies of ensuring drug
296 delivery would be needed. For example, strategies like changes to NP shape or surface
297 chemistry may ensure escape from intracellular vesicles into the more dissolution-
298 friendly conditions of the cytosol (pH 7 - 7.4),³⁹⁻⁴¹ thus avoiding potential NP
299 exocytosis.⁴²

300 At pH 6, an increase in dye release was seen over time for the three NP
301 formulations, although NP_{TEOS} releases FITC at a slower rate than both NP₅₀₋₅₀ and

302 NP_{ETOS} in the first 8 hours (Figure 5b). This is likely due to the more highly crosslinked
303 nature of the core formed solely from TEOS, which results in slower dye diffusion out
304 of NP_{TEOS}. Nonetheless, it is clear that increasing the pH from 4 to 6 led to more rapid
305 dye release from the NPs and is attributed to the increased rate of hydrolysis at higher
306 pH causing particle dissolution.

307 The fluorescence data of the NPs at pH 7.4 clearly showed that dye release due
308 to NP degradation allowed for more rapid release of FITC (Figure 5c). This result
309 correlated well with the electron microscopy results (Figure 2, S3, S4, S5 S6) from which
310 it is evident that extensive particle dissolution occurred after 6 hours. More than 55%
311 of FITC was released from NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS} after 6 hours, which, in the case of
312 oral drug delivery, suggested that small intestine would be the location where the
313 majority of drugs would become available for absorption. This is clearly positive as this
314 would lead to more efficacious delivery of the therapeutic. The loss of dye at pH 7.4
315 may not be beneficial for intracellular delivery as the cargo can be released before
316 localising at tumour sites and prior to endocytosis. The fluorescence data also agrees
317 with the findings of Mahon *et al.* where dye-leaching from SiNPs caused by NP
318 dissolution can occur at physiological pH *in vitro*.³⁴ The authors then developed an
319 alternative SiNP synthetic approach to prevent SiNP dissolution and dye-leaching in *in*
320 *vitro* conditions.

321 Considering the favourable fluorescein retention in acidic conditions and
322 release at higher pH, we decided to investigate whether the microporous SiNPs
323 synthesised in this study may be suited to oral drug delivery. FITC release was
324 monitored over time while increasing the pH, in an attempt to mimic the pH conditions
325 of the whole GI tract and the digestion process (i.e. stomach, pH 4, to duodenum, pH6,
326 to jejunum, pH 7.4, Figure 6a). The results are summarised in Figure 6b as free data
327 points.

328 As expected, at pH 4 the NPs released less than 10% of the FITC cargo over a 4-hour
 329 period. However, when the pH increased to 6 a difference in dye release was observed
 330 for NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS}. NP₅₀₋₅₀ release was higher than NP_{TEOS} and NP_{ETOS} at pH
 331 6 solution. However, the most dramatic trend was the ‘burst release’ profile of FITC
 332 from NP_{ETOS} whilst incubated at pH 6 and pH 7.4. Only 3% of the FITC cargo was
 333 released at pH 4 over 4 hours, but once the NP_{ETOS} experienced small intestine-like
 334 conditions, the rate of release rapidly increased and 70% of dye was released into
 335 solution after 8 hours. The overall release of FITC from NP_{ETOS} was 80% after 12 hours.
 336 While NP₅₀₋₅₀ showed the highest release of FITC at pH 6, no dramatic increase in
 337 release was observed at pH 7.4, with 62% of the loaded FITC was detected in the

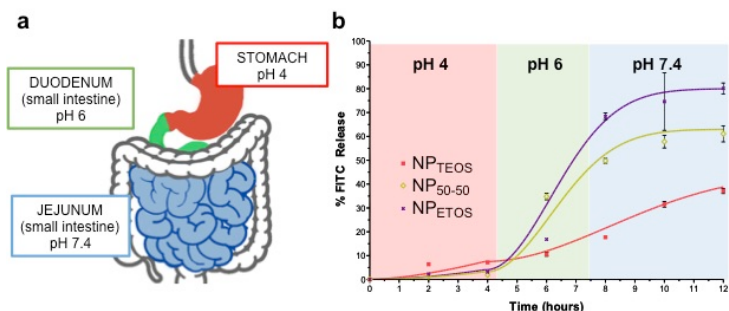


Figure 6: (a) The pathway through the gastrointestinal tract was mimicked over time. Stomach pH (pH 4) refers to that of ‘fed state’ and the requirement for some therapeutics like nonsteroidal anti-inflammatory drugs to be administered concurrently with food ingestion (b) Marginal FITC was released at pH4. Then, a pH-dependent dye release profile was observed for the respective NPs. NP_{TEOS} released FITC slowly and in a sustained manner at pH 6 and pH 7.4. NP₅₀₋₅₀ and NP_{ETOS} displayed initial burst release at pH 6 followed by a steady release at pH 7.4. Greater dye retention in the NPs was observed when increased TEOS was used for core formation. The free data points were used to manually fit Higuchi-Peppas models.

338 supernatant after 12 hours. On the other hand, NP_{TEOS} exhibited slow dye release at pH
 339 6 and pH 7.4 and released less than 40% of its fluorescent cargo after 12 hours.

340 To further understand the FITC release from the NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS}
 341 presented in Figure 6b, the Peppas kinetic model was considered as an appropriate
 342 model to assess diffusion-based cargo release from drug delivery systems.^{43,44} The
 343 model is typically applied to polymeric systems (SI, Equations 1 and 2). The dye release
 344 was simulated for NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS} and the simulated profiles appeared to fit
 345 the experimental observations well. The same rate constants were used for the fitting
 346 of the NP₅₀₋₅₀ and NP_{ETOS} data which suggests the incorporation of ETOS in the NP core
 347 led to similar dye diffusion pathways for the two types of colloid. However, the higher
 348 retention of FITC by NP₅₀₋₅₀ compared to NP_{ETOS} suggested that the former presumably

349 had a more densely formed silica matrix that eventually limited the release of the dye
350 during the time period studied. Different rate constants were needed to fit the NP_{TEOS}
351 data and suggested a different overall FITC release mechanism compared to both NP₅₀₋
352 ₅₀ and NP_{ETOS}. This is consistent with the hypothesis where a higher crosslinking density
353 in a NP core matrix formed from TEOS alone and was reflected by the higher retention
354 of FITC after 12 hours. The results presented in Figure 6b therefore show that
355 increasing the amount of ETOS during NP synthesis would lead to increased cargo
356 release at the pH found in small intestine (i.e. pH 6 and 7.4). This may prove beneficial
357 if a 'burst release' profile is desirable, whereas it would be preferable to employ NPs
358 formulated solely from TEOS for slower molecular release into the small intestine.

359 These findings established that SiNPs exhibit pH-dependent dissolution profiles,
360 and it is possible to synthesise SiNPs that exhibit different cargo release profiles that
361 hold potential in oral drug delivery applications. The ease at which these microporous
362 NPs were synthesised and shown to exhibit different dissolution behaviour suggests
363 that a number of further studies should be performed with encapsulated molecules of
364 various physicochemical properties. We have also previously developed
365 methodologies for extending SiNP storage and long-term stability,^{22,45} and implied that
366 the successful approaches for synthesising microporous SiNP with drug molecules
367 could potentially be developed into realistic nano-delivery systems. In addition, the
368 particles presented here may also be applicable to the emerging field of nano-
369 nutraceuticals;^{1,44,46} a field concerned with tuning molecule release kinetics and
370 absorption using nano-sized carriers for more effective nutrient delivery systems. The
371 use of microporous SiNPs therefore offers a number of potential routes for improved
372 transport, protection and release of therapies in oral drug delivery and indeed the drug
373 delivery field as a whole.

374

375 **Conclusion**

376 Microporous SiNPs with core-shell architecture were synthesised and their dissolution
377 in biologically relevant pH (pH 4, pH 6, pH 7.4) was assessed. These pH refer to those
378 found intracellularly and in the gastrointestinal tract. NP cores were formed using
379 tetraethoxysilane (NP_{TEOS}), ethyl triethoxysilane (NP_{ETOS}) or a 1:1 ratio of both
380 precursors (NP₅₀₋₅₀). These NPs did not degrade in pH 4 conditions but exhibited

381 degradation and fluorescein-release at pH 6 and pH 7.4. This was attributed to
382 accelerated hydrolysis of the silica matrix at higher pH, the formation of mesopore-
383 sized hollows and subsequent NP dissolution. This suggested that dissolution-based
384 cargo release from the NPs presented here may be more likely to diffuse from of the
385 NPs at physiological pH (pH 7.4) before being endocytosed and entering intracellular
386 vesicles (pH 6 – early endosome, pH 4 - late endosome/lysosome). The degradation of
387 the NPs at pH 7.4 also infers that this class of nanomaterial could be safely cleared and
388 excreted. On the other hand, the retention of the fluorescein cargo in acidic conditions
389 meant the NPs could be applicable to oral drug delivery where drugs required
390 protection in the stomach. In a mimicked gastrointestinal tract study, increasing the
391 amount of ETOS in the NP core formation led to increased release of FITC in pH 6 and
392 pH 7.4 solutions. The release profiles of FITC are consistent with the hypothesis that
393 cargo release from the NPs is controlled in part by the crosslinking density of the silica
394 core, with ETOS generating a less dense matrix that facilitates greater cargo release at
395 small intestinal pH (pH 6 and pH 7.4). The data obtained for NP_{TEOS} suggests this class
396 of SiNP would be more suited to slow drug release in oral drug delivery applications.
397 Overall, while further studies are needed to elucidate the degradation mechanisms
398 associated with the colloidal systems presented here, we showed that it was possible
399 to tune the release of encapsulated from SiNPs by simply changing the precursor used
400 during NP synthesis. Microporous SiNPs therefore hold potential as a flexible platform
401 upon which to base oral drug delivery strategies.

402

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Supporting Information

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519 **pH-Dependent Silica Nanoparticle Dissolution and Cargo Release**

520

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544 **Additional experimental information**

545 Materials

546 Cyclohexane (anhydrous, 99.5%), 1-hexanol (anhydrous, 99%), Triton® X-100,
547 aminopropyl trimethoxysilane [APTMS] (97%), tetraethoxysilane[TEOS] (99.99%),
548 ethyltriethoxysilane (96%)[ETOS], 4,4'-Bis(triethoxysilyl)biphenyl (95%) [bis(TE)PP], 4,4'-
549 Bis(triethoxysilyl)benzene (96%) [bis(TE)B], ammonium hydroxide solution (28% w/v in
550 water, ≥99.99%), 3-(trihydroxysilyl)propyl methylphosphonate monosodium salt (42%
551 w/v in water) [THPMP], fluorescein isothiocyanate isomer I (≥90%)[FITC], sodium
552 phosphate dibasic (>98.5%), sodium phosphate monobasic (>98%), sodium carbonate
553 (≥99.5%), sodium bicarbonate (≥99.5%), were purchased from Sigma Aldrich. Sodium

554 carbonate (0.1M) combined with sodium bicarbonate (0.1M) yielded pH10.6 (9:1 v/v
555 respectively) solutions. Absolute ethanol, transparent Nunc Maxisorb 96 well plates
556 were purchased from Fisher Scientific. Carbon Films on 400 Mesh Grids Copper were
557 purchased from Agar Scientific.

558 Nanoparticle synthesis

559 Dye precursor formation: In a dried glass vial, FITC (2.5 mg) was dissolved in 1-hexanol
560 (2mL) with APTMS (5.6 μ L). The reaction was stirred for 2 hours under a nitrogen
561 atmosphere.

562 All nanoparticles were formed in a microemulsion prepared by combining
563 cyclohexane (7.5 mL), 1-hexanol (1.133 mL), Triton[®] X-100 (1.894 g) and DI water
564 (0.48 mL) in a 30 mL plastic bottle under constant stirring. For the formation of the
565 silica core, TEOS and ETOS were added in different ratios with quantity of oxysilane
566 being equal 0,45 mmol.

567

568

569

570

	TEOS % (μ L)	ETOS % (μ L)
NP _{TEOS}	100% (100)	/
NP ₅₀₋₅₀	50% (50)	50% (48)
NP _{ETOS}	/	100% (97)

571

572 Dye precursor solution (0.162 mL) was then added. After 30 minutes, 40 μ L of
573 ammonium hydroxide was added to trigger polymerisation. The mixture was stirred
574 for further 24 hours. Nanoparticle shells were synthesised by adding 50 μ L of TEOS. 20

575 minutes later 40 μ L THPMP was added. After 5 minutes, 10 μ L of APTMS was then
576 added, and the mixture was allowed to stir at RT for another 24hrs. The microemulsion
577 was then broken by adding 30 mL ethanol. Formed SiNPs were purified by
578 centrifugation (14000 rpm, 10 min) and re-dispersion in ethanol (x3). After purification,
579 the NPs were stored in ethanol at 4°C.

580 Quantification of FITC loading

581 In order to quantify the amount of FITC loaded during the synthetic procedure, 200 μ g
582 of each type of SiNPs were shaken (600 rpm) at 37°C in sodium carbonate/sodium
583 bicarbonate (1:9) buffer at pH10.6 as previously reported.²¹ After 5 hours, the samples
584 were centrifuged (14000 rpm, 10 min) and no pellet was observed, meaning that the
585 particles had dissolved. Three wells of the 96-well plate were filled with 200 μ L of the
586 supernatant isolated after centrifugation. The signal given by FITC molecules free in
587 solution was compared to a fluorescence/absorbance-based calibration curve of
588 known concentrations of FITC at pH10.6. The amount of dye loaded in 200 μ g of
589 particle were calculated. From the values obtained, the number of molecules per NP
590 was calculated by using the spherical volume of the silica NPs calculated from average
591 TEM diameters. The signal was read at 490/525 nm ($\lambda_{ex}/\lambda_{em}$). Values are reported as
592 average of three independent batches of particles (n=3) \pm SD.

593

594

595 Synthesis of NPs using benzene-oxysilanes

596 The same microemulsion and FITC-loading setup as described above was used except
597 for the choice of oxysilanes. Again, a total of 0.45mmol of oxysilane was used. TEOS
598 was used for NP formation alongside either bis(triethoxysilyl)benzene [bis(TE)B] or
599 bis(triethoxysilyl)biphenyl [bis(TE)PP].

TEOS:Bis(TE) B	TEOS [μL]	Bis(TE)B [μL]
95:5	95	8.92
90:10	90	17.85
85:15	85	26.77
75:25	75	44.62
50:50	50	89.24
TEOS:Bis(TE) PP	TEOS [μL]	Bis(TE)PP [μL]
75:25	75	51.44
50:50	50	102.88

600

601 Nanoparticle shells were synthesised by adding 50 μL of TEOS, followed by 40 μL of
602 THPMP and 10 μL of APTMS after 20min and 5min between each other. After 24h, the
603 microemulsion was broken by adding 30 mL ethanol. Formed SiNPs were purified by
604 centrifugation (14000 rpm, 10 min) and re-dispersion in ethanol (3x). After purification,
605 the nanoparticles were stored in ethanol at 4°C.

606 Buffer preparation

607 Phosphate buffer at different pH were prepared mixing 0.2 M sodium phosphate
608 dibasic and 0.2 M sodium phosphate monobasic and adjusting the pH to 4, 6 and 7.4
609 using 5 M NaOH and 5 M of HCl.

610

611 NP characterization

612 *Dynamic light scattering and zetametry:* SiNPs were dispersed at a concentration of
613 500µg/mL in DI water. Their size and zeta-potential were analysed in a disposable
614 folded capillary cell (DTS1070) at RT using Malvern Zetasizer. n = 3, average ± SD.

615 *SiNP stability:* 250µg/mL of NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS} were isolated and re-dispersed
616 in 1mL of each buffer (pH 4, 6 and 7.4) and incubated at 37°C. Size and zeta potential
617 were measured by DLS at 0hr, 24hr, 48hr using Malvern Zetasizer. n = 3, average ± SD.

618 *Transmission electron microscopy:* NP size quantification following synthesis: 5µL of
619 NPs in water (500µg/mL) was added on 'Carbon Films on 400 Mesh Grids Copper'
620 (Agar Scientific) and allowed to evaporate. Using ImageJ software, at least 100 NPs
621 per image were analysed for NP diameter.

622 *SiNPs dissolution using TEM:* Following incubation in pH 4, 6, or 7.4 over different
623 times, NP pellets were isolated using centrifugation (x3), washed using DI water in
624 order to remove residues salts. The pellet was finally re-dispersed in 200µL DI water,
625 3µL added to 'Carbon Films on 400 Mesh Grids Copper' (Agar Scientific) and allowed
626 to evaporate. Images were taken on a Joel JEM-3200FS at ×250, ×200, ×150 and ×100
627 magnification.

628

629 *Scanning Transmission Electron Microscopy (STEM): SiNPs dissolution:* The same grids
630 as 'SiNPs dissolution using TEM' prepared for TEM analysis for the main text were used
631 for STEM. The grids were analysed in STEM imaging mode using a Hitachi SU-6600
632 microscope. Images were taken in secondary electron (SE) and transmission electron
633 (TE) mode at 130,000 magnification using either 20kV or 25kV accelerating voltage.
634 The working distance was 8mm.

635

636 Data fitting of with Peppas model for data points in Figure 6b

637 The data from the release profile of the 'GI tract-like assay' was manually simulated
638 with SigmaPlot using the diffusive models presented by Siepmann and Peppas.⁴³
639 Equation 1 was used to fit data from 0 to 4 hours.

640 $\frac{M_t}{M_\infty} = (k_{s1})(\sqrt{time}) + (k_{s2})(time)$

641 [Eq. 1]

642 where M_t is the diffused mass at a given time, M_∞ is the asymptotic diffused mass at
 643 infinite time, k_{s1} and k_{s2} are diffusive and relaxation constants. Equation 2 was used to
 644 fit the data from 4 to 12 hours.

645 $\frac{M_t}{M_\infty} - \frac{M_4}{M_\infty} = (k_{i1})(\sqrt{time - 4}) + (k_{i2})(time - 4)$

646 [Eq. 2]

647 where M_4 is the predicted diffused mass at the time of changing from pH4 to pH6 (i.e.
 648 after 4 hours). The rate constants used to for Equation 1 and 2 are presented below.
 649 M_∞ for NP_{TEOS}, NP₅₀₋₅₀ and NP_{ETOS} were 45, 63 and 80 respectively.

	NP _{TEOS}	NP ₅₀₋₅₀	NP _{ETOS}
k_{s1}	0.02	0.0025	0.0025
k_{s2}	0.01	0.005	0.005
k_{i1}	0.01	0.001	0.001
k_{i2}	0.05	0.2	0.2

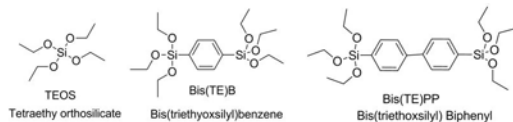
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653 **Supporting Figures**

654



Rapid visual aggregation in DI water in less than 1 minute.

In ethanol		Z-average (nm)	Zeta Potential (mV)
TEOS : Bis(TE)B	75:25	170.1 ± 12.0	-20.6 ± 1.6
	50:50	177.3 ± 8.4	-29.8 ± 2.4
TEOS : Bis(TE)PP	75:25	179.7 ± 16.2	-5.8 ± 0.3
	50:50	180.7 ± 8.7	-9.3 ± 0.5

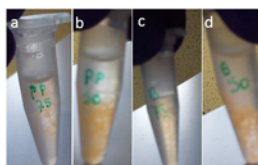
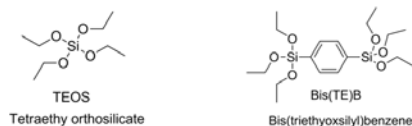


Figure S1: FITC-doped silica NPs were formed by combining the traditional precursor TEOS and either bis(triethoxysilyl)benzene, Bis(TE)B, or bis(triethoxysilyl)biphenyl, Bis(TE)PP. The ratio TEOS:Bis(TE)B and TEOS:Bis(TE)PP was 75:25 and 50:50. The resultant colloids were soluble in ethanol and dynamic light scattering was used to quantify the diameter and zeta potential of the NPs (n=3), as shown in the above table. However, when they were dispersed in DI water the NPs visually aggregated in less than 1 minute. [a , b: TEOS:Bis(TE)B 75:25, 50:50]; c, d: TEOS:Bis(TE)PP]

655

656

657



Visual aggregation in phosphate buffer saline in less than 10 minutes.

In water		Z-average (nm)		Zeta Potential (mV)	
		0 hours	24 hours	0 hours	24 hours
TEOS:Bis(TE)B	95:5	109.4 ± 3.8	97.7 ± 3.1	-32.1 ± 0.7	-31.9 ± 0.4
	90:10	114.5 ± 4.6	107.4 ± 4.8	-37.6 ± 1.2	-22.1 ± 3.1
	85:15	233.4 ± 19.6	231.0 ± 20.2	-31.1 ± 1.1	-14.1 ± 0.5

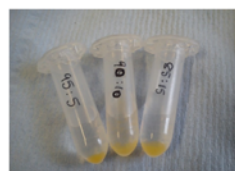


Figure S2: Bis(TE)B was incorporated in to FITC-loaded silica NPs in lower molar concentrations as a way make the resultant NPs 'less hydrophobic' and therefore stable in aqueous conditions. TEOS:Bis(TE)B was added to the microemulsion in 95:5, 90:10, 85:15 and were colloidally stable in DI water for 24 hours. However when the NPs were dispersed in PBS they visually aggregated after only 10 minutes.

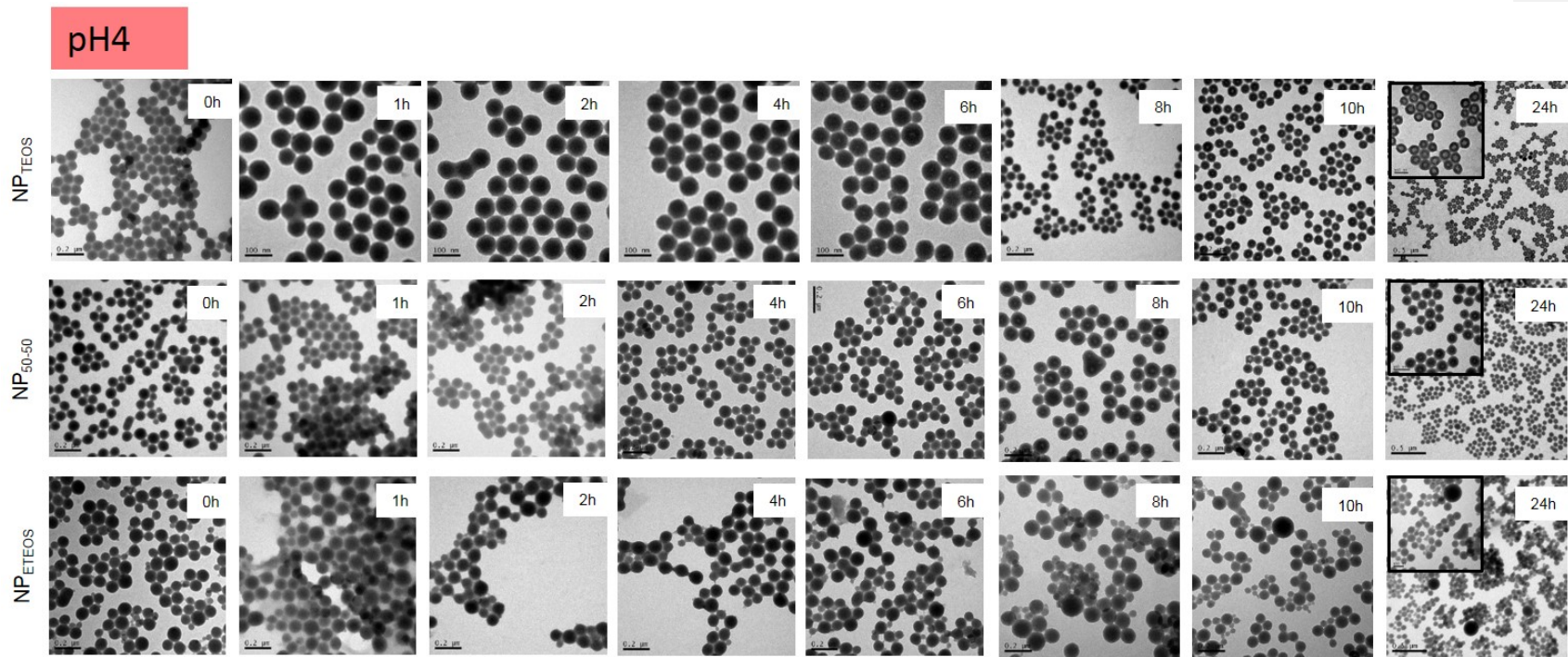


Figure S3: The three sets of NPs appeared in tact when incubated over time in pH 4

pH 6

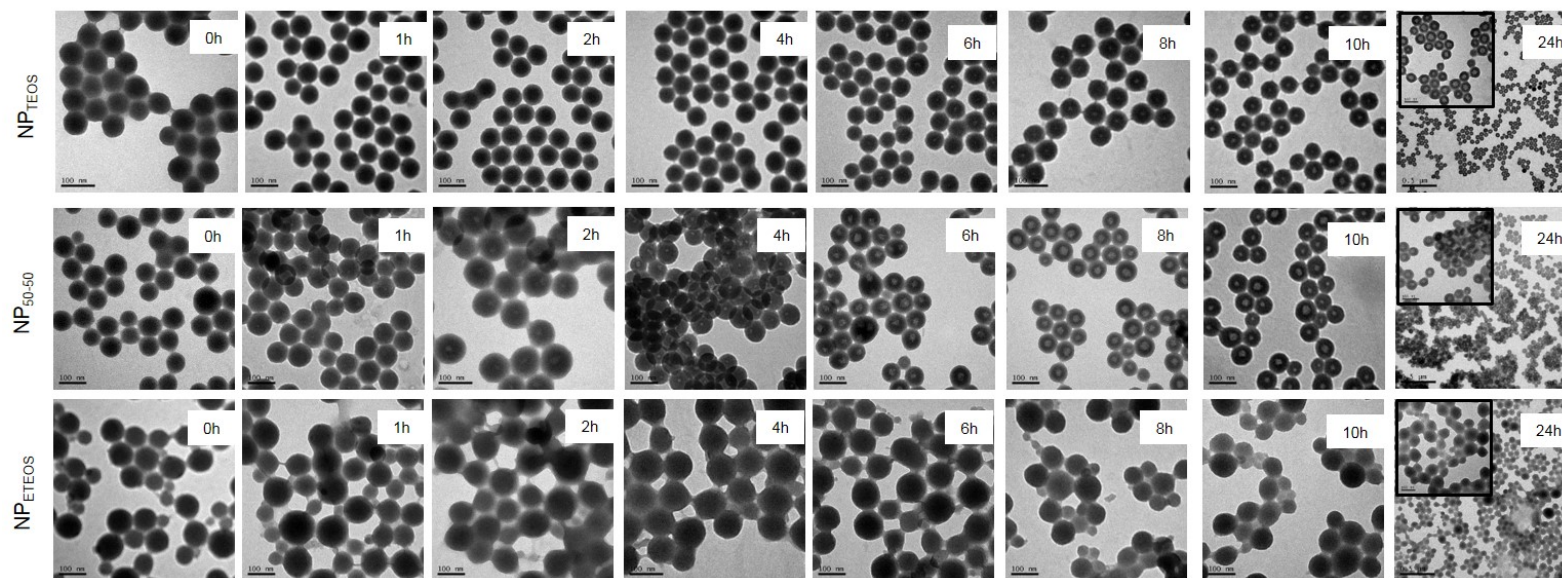


Figure S4: Degradation was visible by TEM for the three sets of NPs in pH 6 solution over time. Hollowing in the interior of NP_{TEOS} and NP₅₀₋₅₀ was observed after 6 – 8 hours whereas NP_{ETEOS} appeared to degrade at the particle surface.

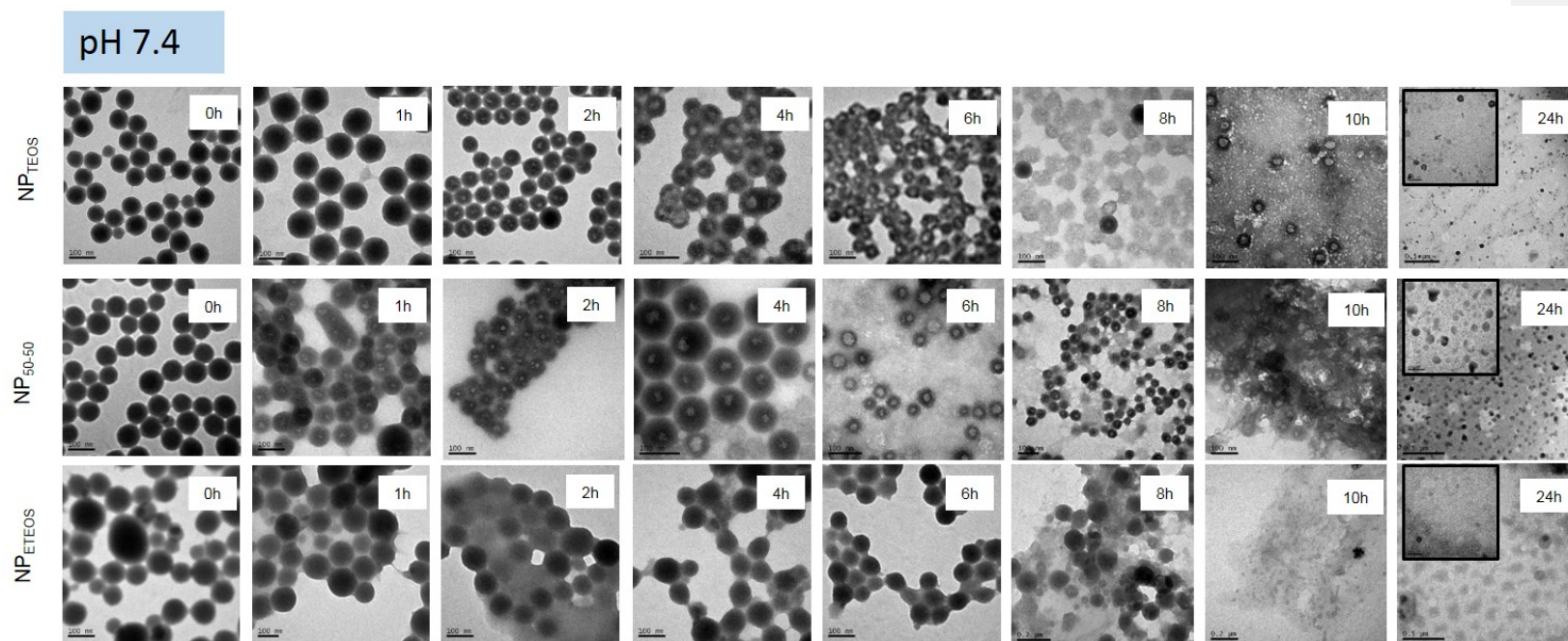


Figure S5: Degradation was visible by TEM for the three sets of NPs in pH 7.4 solution over time. More rapid hollowing of the interior of NP_{TEOS} and NP₅₀₋₅₀ was observed compared to those observed at pH 6. After 6 – 8 hours few NPs could be isolated after centrifugation and those after that time. NP_{TEOS} appeared to degrade at the particle surface. After 10 hours virtually no NPs were visible by TEM, and structures resembling colloids were highly degraded and surrounded by dissolution debris.

Table S3: The size of the NP hollows (or pores) where measured by TEM analysis using the micrographs from Figures S3, S4 and S5.. NM (not measurable) indicates that the particles did not present any visible pores, while DISS (dissolved) indicates that no particles were identifiable on the TEM grid and were therefore considered to be dissolved. Values are shown as average \pm SD (n=30 approximately).

		0h	1h	2h	4h	6h	8h	10h	24h
pH4	NP _{TEOS}	NM	NM	NM	NM	9.65 \pm 5.30	7.79 \pm 3.33	14.11 \pm 4.85	32.84 \pm 7.69
	NP ₅₀₋₅₀	NM	NM	NM	NM	NM	14.98 \pm 6,79	12.77 \pm 5.65	11.41 \pm 4.25
	NP _{E_{TEOS}}	NM	NM	NM	NM	NM	NM	NM	NM
pH6	NP _{TEOS}	NM	NM	NM	NM	6.96 \pm 3.73	8.07 \pm 2.63	11.46 \pm 3.37	34.01 \pm 8.66
	NP ₅₀₋₅₀	NM	7.52 \pm 2.19	13.71 \pm 4.93	11.11 \pm 3.18	16.39 \pm 5.50	24.61 \pm 6.16	20.31 \pm 7.32	26.91 \pm 6.17
	NP _{E_{TEOS}}	NM	NM	NM	NM	NM	NM	NM	NM
pH7.4	NP _{TEOS}	NM	NM	11.76 \pm 3.43	13.45 \pm 4.93	4.80 \pm 1.44	DISS.	DISS.	DISS.
	NP ₅₀₋₅₀	NM	15.39 \pm 5.38	14.91 \pm 4.80	20.45 \pm 6.50	21.16 \pm 7.2	13.35 \pm 4.40	DISS.	DISS.
	NP _{E_{TEOS}}	NM	NM	NM	NM	NM	NM	DISS.	DISS.

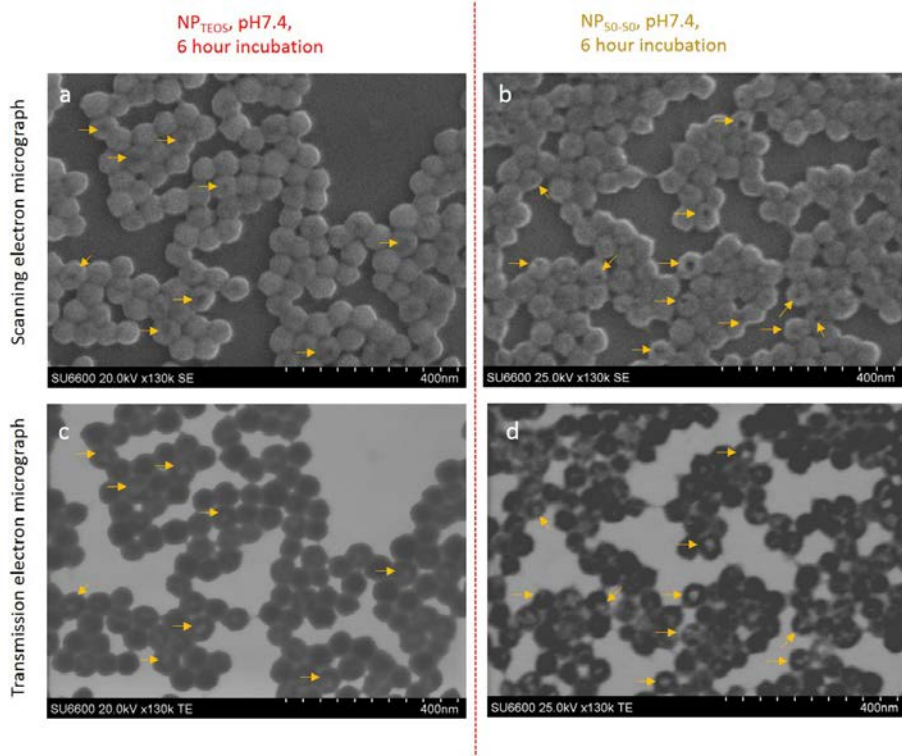


Figure S6: Scanning transmission electron microscopy allowed for secondary electrons (SE) to be obtained for scanning mode while transmission electrons (TE) could be detected simultaneously in transmission mode. (a,b) Scanning electron micrographs showed that the surface deformations, highlighted by yellow arrows, were visualised as hollows in transmission electron micrographs (c,d). It is therefore suggested to that studies investigating silica NP hollowing/etching of the core should also use scanning electron microscopy to interrogate the particle surface, thus providing a more accurate evaluation of the overall particle morphology and integrity.