

Graphene, graphene oxide and reduced graphene oxide as pH sensitive sensor materials

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Abstract

pH measuring and monitoring is fundamental to understand or control many chemical processes in biological, industrial or environmental fields. The state-of-the-art pH sensor is the glass electrode, but single-use paper strips are also widely used and there are also a few ion-selective field effect transistors (ISFETs). Due to the excellent properties of graphenic materials, new pH sensors can be fabricated that fulfil the increasing request for lightweight, miniaturized and cost-effective products. This review describes how graphene, graphene oxide and reduced graphene oxide can be used as sensitive materials for pH sensors. The various configurations are reported along with the advantages and current limitations.

Introduction

29 pH governs many chemical reactions in nature. Biology, environmental protection, food
30 quality control, medicine and industry are just a few fields where pH sensors have been
31 extensively used to understand the nature of chemical processes, as well as how to monitor
32 the quality and control the safety¹⁻⁶. Although paper test strips and glass electrodes remain
33 the most widespread pH sensors, many studies have focused on developing less fragile,
34 miniaturized, and biocompatible sensors, with a better signal-to-noise ratio (SNR). The pH
35 signal can be transduced through various techniques and technologies, e.g. potentiometry,
36 optics, and ion-selective field effect transistors (ISFETs).

37 Illustrative pH-sensitive materials reported in the literature include polyaniline (PANI),
38 polypyrrole (PPy), polyparaphenylenediamine, poly(vinyl pyridine), poly(methacrylic acid)
39 (PMAA), poly(acrylic acid) (PAA), palladium oxide, aluminium oxide, riboflavin,
40 anthraquinonesulfonate, fluorescent dyes such as rhodamine, silicates and silanes, 6-
41 carboxyfluorescein, as well as seminaphthorhodafluor (SNARF)/seminaphthofluorescein
42 (SNAFL), hydrogels such as hydroxyethyl methacrylate/dimethylaminoethyl methacrylate
43 (HEMA-DMAEMA) and poly(vinyl alcohol)/poly(acrylic acid) (PVA-PAA)⁷⁻²⁷.

44 Despite the high number of materials and devices reported in the literature, only a few have
45 been marketed. The most challenging hurdles are the degradation of materials, low
46 sensitivity, drift and difficulties in mass production. ISFETs are currently the most effective
47 alternative to glass electrodes. Stable and low drift pH-ISFETs suitable for long use are on
48 the market, such as the DuraFET[®] sensor by Honeywell. However, although the DuraFET[®]'s
49 sensing element is only a few millimetres wide, the probe is longer than 15 cm and
50 impractical, for example, for *in vivo* biosensing²⁸. A multi-purpose and high performance
51 micro/nano sensor is the holy grail for pH measurement.

52 After the isolation of graphene from highly oriented pyrolytic graphite in 2004 at the
53 University of Manchester²⁹, many researchers started to investigate how to exploit the
54 extraordinary electrical, chemical, mechanical and optical properties of this material for

55 sensing and biosensing³⁰⁻³³. The first section of this review focuses on the chemico-physical
56 properties and mechanisms that underlie graphene's sensitivity to pH as well as pH sensors
57 exploiting such properties.

58 Graphene oxide (GO) resulting from the oxidation of graphite is the precursor of reduced
59 graphene oxide (rGO), i.e. graphene obtained by the exfoliation and reduction of GO³⁴. GO
60 sensitivity to pH depends on the surface hydroxyl (OH), carboxyl (COOH) and epoxy groups
61 (COC) that protonate/de-protonate when pH changes: GO and its applications for pH
62 sensing are discussed in the second section of this review. Although reduced graphene
63 oxide shares many of graphene's physical and chemical properties, there are differences
64 such as the presence of defects and residual oxygen atoms not removed by reduction³⁵ that
65 justify discussion in a separate section. In the literature, graphene, GO and rGO are often
66 mixed with other compounds to obtain pH sensitive materials, mainly to improve the
67 mechanical structure or the electrical conductivity of the resulting composite.

68 This review only reports devices where graphene, GO or rGO are used as the main pH
69 sensitive element. We provide an overview of research efforts in the last decade and trends
70 for the development and enhancement of graphene-based pH sensors.

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74 **Graphene**

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76 **Solution-gated ISFETs**

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78 A typical configuration of a graphene-based ISFET is the solution-gated FET (SGFET),
79 where the graphene is used as the conduction channel. The SGFET resembles a three-
80 electrode electrochemical cell, where the electrolyte solution is in direct contact with

81 graphene (working electrode), the gate voltage (V_G) is applied through a reference electrode
82 (Ag/AgCl, calomel or Pt), and a Pt counter electrode completes the device. Although the
83 counter electrode is often absent, this top-gated FET with Ti/Au or Cr/Au contacts for source
84 and drain is a common configuration for pH measurement (Fig. 1a). The most common
85 methods for fabricating the graphene channel are the exfoliation of graphite and the epitaxial
86 growth, typically by chemical vapour deposition (CVD). After the CVD of carbon atoms on a
87 substrate such as copper, the graphene film is coated with a protective material, e.g.
88 poly(methyl methacrylate) (PMMA) or poly(dimethylsiloxane) (PDMS), then the substrate is
89 etched and the film is transferred onto the ISFET substrate, e.g. SiO_2/Si or 6H-SiC.

90 In 2008, Ang *et al.* proposed an SGFET to measure pH including an epitaxial graphene layer
91 grown on 6H-SiC³⁶. Ang *et al.* hypothesized conductivity (σ) of the graphene channel to be
92 modulated by the adsorption of hydroxyl (OH^-) and hydroxonium (H_3O^+) ions onto the
93 surface. They used a $V_G \in [-1, 1]$ V and a constant drain–source voltage (V_{DS}) of -1 V, and
94 observed that OH^- bound at the inner Helmholtz plane of the graphene/solution interface
95 attracted their counterions in graphene in the case negative V_G , thus inducing a p-type
96 doping. On the other hand, n-type doping was induced by the H_3O^+ adsorption in the case
97 of positive V_G or acid pH. As pH increased from 2 to 12, the Dirac voltage (V_{Dirac} , i.e. the
98 voltage at which the number of holes and electrons is balanced and the channel
99 conductance is minimum) showed a positive shift (Figs. 1b, 1c), thus allowing the pH to be
100 measured. Surprisingly, for negative V_G and 3-4 graphene layers, a sensitivity of about 99
101 mV/pH was found, which is greater than the Nernst limit of 59.16 mV/pH.

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103 Lei *et al.* fabricated a chemiresistor, i.e. a gate-free FET, with a graphene sheet exfoliated
104 from graphite and deposited onto a SiO_2/Si substrate³⁷. For a constant current of 10 μA and
105 an initial resistance of 86 K Ω , the sensitivity of the device from pH 4 to 10 was approximately
106 2.13 K Ω /pH. The resolution was calculated as the ratio between the maximum standard

107 deviation of resistance for multiple measurements and the sensitivity of the device. The
108 resolution at basic pH and acid pH were 0.33 and 0.97, respectively. This behaviour may be
109 explained assuming that the OH^- are more ordered on the inner Helmholtz plane than the
110 H_3O^+ . This sensor was reusable despite a hysteresis of about 2 K Ω . The authors suggested
111 that reusability was probably related to the easy removal of electrolytes from graphene due
112 to its extremely low thickness, although the paper did not report any precise value or further
113 explanation. Lee *et al.* proposed another example of a gate-free FET, this time on a paper
114 substrate³⁸. Graphene was dispersed in sodium dodecyl sulfate (SDS) and, after sonication,
115 the dispersion was poured onto a paper substrate through a metal mask under vacuum.
116 After rinsing with deionized water and drying at 80 °C for 20 min, six U-shaped graphene
117 sensors were fabricated. A paraffin pen was used to define a closed measurement area
118 around each sensor. The highest fabrication repeatability and electrical stability were
119 obtained by depositing 0.5 mg of graphene dispersion through vacuum filtration, which
120 resulted in a sensing layer thickness of about 100 μm . The response was measured from
121 pH 1 to about 10.5 by dispensing 10 μL drops of different buffer solutions in each of the six
122 measurement areas. At $V_{\text{DS}} \in [-1, 1]$ V, the sensitivity was about 30.8 Ω/pH and the
123 correlation between resistance and pH resulted in a regression line with a coefficient of
124 determination $R^2 \cong 0.93$.

125 Following the same structure as Fig. 1a, Ohno *et al.* described a FET with a single layer of
126 graphene and a leakage current lower than 10 nA³⁹. The conductance was measured for
127 two FET configurations: top-gate and back-gate, where the back-gate is an n-doped Si
128 substrate. The top-gate capacitance was three times greater than in the back-gate
129 configuration, therefore confirming the top-gate as the preferred option for sensing. The
130 V_{Dirac} positively and linearly shifted at increasing pH from 4 to 8.2. For $V_{\text{G}} \in [-50, 50]$ mV, the
131 sensitivity was about 30 mV/pH. The authors pointed out that the V_{Dirac} may shift at different

132 pH values because of charged impurities scattering, which can be caused, for example, by
133 the residual resist used in the photolithographic fabrication process, or graphene defects.
134 Ristein *et al.* used a monolayer of graphene on a 6H-SiC single crystal⁴⁰. The solution-gated
135 FET (SGFET) had a leakage current lower than 1 nA and linear I-V characteristics up to
136 $|V_{DS}| = 0.1$ V. The drain-source current (I_{DS}) versus V_G was measured at $V_{DS} = -50$ mV from
137 pH 3 to 12. The V_{Dirac} shift per unit pH, i.e. the sensitivity, was 19 ± 1 mV/pH. The authors
138 compared the conductivity of the graphene channel (3.5×10^{-5} S) with the value reported by
139 Ang *et al.* (3.5×10^{-4} S) since their transistors were similar. The difference of one order of
140 magnitude in conductivity was probably because Ang *et al.* tested their SGFET at $V_G > 0.3$
141 V, which Ristein *et al.* found to be the maximum voltage that would prevent defects in the
142 graphene layer. These defects probably caused the formation of holes, which then led to a
143 super-Nernstian sensitivity.

144 Cheng *et al.* etched an SiO₂ substrate to suspend a graphene layer shorter than 1 μ m
145 between the Cr/Au contacts of drain and the source⁴¹. The resulting FET led to a V_{Dirac} of
146 0.1 ± 0.2 V and to a larger conductivity at negative V_G (majority of holes) compared to
147 positive V_G (majority of electrons). Compared with a standard configuration, there was a net
148 improvement in the FET characteristics with the suspended graphene: the SNR increased
149 by 14 dB at frequencies below 1 KHz, and the sensitivity increased 1.5 and 2 times for holes
150 and electrons, respectively, leading to a symmetric σ - V_G curve. These improvements were
151 ascribed to a reduced scattering, but a full explanation was not supplied. This FET had a
152 sensitivity of about 17 mV/pH when tested with buffer solutions from pH 6 to 9 and $V_G \in [-$
153 50, 50] mV.

154 Studies on the effects of different electrolyte solutions have further contributed to the
155 comprehension of the mechanisms regulating the graphene response to pH. Lee *et al.*
156 investigated the V_{Dirac} shift when an SGFET was exposed to sodium hydroxide (NaOH),
157 hydrochloric acid (HCl) and potassium hydroxide (KOH)⁴². A graphene film was grown onto

158 a copper film in a furnace at 1000 °C in presence of a mixture of CH₄ and H₂. PMMA
159 dispersed in chlorobenzene was used to coat and transfer the graphene layer onto a Si/SiO₂
160 wafer after copper etching. The wafer had pre-patterned source, drain and Cr/AU electrodes.
161 PMMA was removed by rinsing the device with a solution of acetone and deionized water,
162 whereas graphene was patterned with standard photolithographic techniques. Solutions at
163 different pH values in the range [6, 8] were obtained with different concentrations of Na⁺, Cl⁻
164 and K⁺ in 0.05 M phosphate solutions (Table 1).

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166 While the SGETs reported in other works had positive V_{Dirac} shifts at increasing pH, Lee *et*
167 *al.*'s transistor showed a negative V_{Dirac} shift which depended on the ionic composition and
168 strength of the buffer solutions. At $V_{\text{DS}} = 50$ mV, $V_{\text{G}} \in [0, 1]$ and for pH 6, 7 and 8, the pH
169 sensitivities were -78, -38 and -7 mV/pH in buffer solutions containing different
170 concentrations of Na⁺, Cl⁻ and K⁺ respectively, whereas the sensitivity was +69 mV/pH in
171 reference buffers (0.05 M). Therefore, a negative shift for increasing pH was observed in the
172 lab-made buffers containing the electrolytes and a positive shift in commercial reference
173 buffers. This result could not be explained in terms of ionic strength as the response for
174 sodium and potassium buffers were very different, although the ionic strengths were
175 identical. The type of ion played an important role, as the V_{Dirac} shift was more sensitive to
176 the concentration of Na⁺ than to the concentrations of Cl⁻ or K⁺. Lee *et al.* hypothesized that
177 the shift in V_{Dirac} was a consequence of the ionic charge screening, which depends on ion
178 size, degree of hydration, and affinity towards the graphene surface. They suggested pH
179 measurements should only be performed in diluted solutions, as ionic charge screening is
180 more significant at high concentrations. In fact, their tests with five and ten times diluted
181 sodium solutions yielded the typical positive V_{Dirac} shift for increasing pH, whereas the sensor
182 increased its sensitivity from -7 to -27 mV/pH in a 0.1 M potassium solution.

183 Heller *et al.* expanded this research by using different KCl concentrations buffered at pH 7
184 and demonstrating a linear dependence between the V_{Dirac} shift and the $\log(\text{KCl})$
185 concentration with respect to the V_{Dirac} normalized to its value at 1 M KCl in the range [1 mM,
186 1 M]⁴³. Similar results were obtained with LiCl at pH 3. At high ionic strength, the double
187 layer capacitance at the interface graphene-buffer increases and the surface potential
188 decreases. At pH 7, the V_{Dirac} shift changed by -42.7 mV/decade with the buffer
189 concentration. At pH 3, there was a sign inversion (+18.9 mV/decade), probably because of
190 the supporting SiO₂ layer. In fact, SiO₂ has ionized silanol and silylamine groups, such as
191 SiO⁻, SiOH₂⁺ and SiNH₃⁺, which can negatively or positively charge the surface. The effect
192 of different ions on the measurement of pH was confirmed by testing Li⁺, K⁺ and
193 tetraethylammonium (TEA⁺), but an explanation of this effect has still to be found. However,
194 Heller *et al.* hypothesized that the ionic radius or a specific adsorption onto graphene could
195 play a major role.

196 Fu *et al.* (ref. 44) pointed out that the range of graphene sensitivities to pH reported in the
197 literature was very large (12–99 mV/pH). They thus set up an experiment with the three-
198 electrode configuration shown in Fig. 1a. Graphene was exfoliated or grown on copper by
199 chemical vapour deposition and then transferred to the SiO₂/Si substrate. For $V_{\text{DS}} \in [10, 50]$
200 mV and $V_{\text{G}} \in [0.1, 0.8]$ V, the leakage current (1 nA) was negligible compared with the
201 measured I_{DS} . Although the V_{Dirac} shifted positively at increasing pH (as in previous works),
202 the sensitivity was only 6 ± 1 mV/pH from pH 5 to 10. The hydrophobicity of the graphene
203 surface was then increased by adding fluorobenzene for 30 s and drying the FET. In this
204 case, the sensitivity was almost zero from pH 4 to 10. On the other hand, when an Al₂O₃
205 layer thinner than 2 nm was deposited (atomic layer deposition, 100 °C) on graphene, the
206 sensitivity increased to 17 mV/pH from pH 3 to 8. According to Fu *et al.*, OH functional
207 groups from Al₂O₃ turned graphene partially hydrophilic. Their conclusion was that a defect-
208 free graphene is not able to sense pH, and that graphene response to pH depends on the

209 presence of imperfections, such as the hydroxyl and carbonyl groups. The sensitivities of 99
210 and 20 mV/pH reported in (ref. 36) and (ref. 41), respectively, probably depended on a high
211 leakage gate current.

212 Kwon *et al.* investigated the role of defects in graphene mesh FETs (GM-FETs)⁴⁵. The mesh
213 structure was synthesized using silica spheres as a growth mask to avoid photolithographic
214 patterning, thereby reducing the disorder and contamination of carbon atoms. A hexagonal
215 close-packed monolayer of silica spheres covered a copper foil as well as the graphene
216 mesh aroused from the dissociation of carbon atoms at the copper–silica interface. The
217 resulting mesh was a hexagonal array of circular holes with an average diameter and
218 interspacing of 300 and 600 nm, respectively. At different pH values from 6.55 to 8.25 and
219 with $V_{DS} = 50$ mV, the GM-FETs had a sensitivity of about 90 mV/pH. The authors suggested
220 that this super-Nernstian sensitivity could depend on a high number of unsaturated carbon
221 atoms at edge defects, which could adsorb or probably bind the H^+ ions. Although the
222 formation of covalent bonds improved sensitivity, the irreversibility of this reaction could limit
223 the use of GM-FETs. In fact, sensitivity decreased to about 7 mV/pH after five cycles of 3
224 min tests of the GM-FETs in solutions at pH 8.25, 7.85, 7.40, 6.95, and 6.55. Therefore, it is
225 essential to control graphene defects in order to obtain reproducible and reliable results.

226 As the edges of graphene are chemically reactive, Tan *et al.* patterned the graphene layer
227 by electron-beam lithography (EBL) and oxygen plasma into four columns of 99.1 ± 1.5 nm
228 wide and 5.5 μ m long nanoribbons (GNRs) to improve the number of hydroxyl groups⁴⁶.
229 With a constant $V_{DS} = 10$ mV, sensitivity improved after patterning from about 6.5 to 23.6
230 mV/pH in the pH range [6, 8]. One advantage of the edges defects is that unlike defects in
231 the basal plane they do not affect the current flow in the graphene channel. Tan *et al.* also
232 found that the improvement did not depend on impurities such as residual PMMA used in
233 the photolithographic process, since similar results were obtained when hydrogen
234 silsesquioxane (HSQ) was used as the photoresist.

235 Zhang *et al.* combined the ideas exposed in (refs 41, 46) in order to improve the graphene
 236 sensitivity to pH and fabricate a suspended GNR ISFET for measuring pH and cancer
 237 markers in solution⁴⁷. In this study, the width of GNRs was 50 nm and the GNR ISFET was
 238 powered with $V_{DS} = 1$ V, whereas $V_G \in [-1,8, 1.8]$ V. The I_{DS} was normalized with respect to
 239 the current I_0 at pH 9 and presented as the ratio $(I_{DS} - I_0)/I_0$. From pH 5 up to 9, the suspended
 240 GNR FET was 1.5 times more sensitive (about 25 mV/pH) than an unsuspended GNR FET
 241 and a normal SGFET. However, as reported in (ref. 40), those voltages could lead to the
 242 formation of defects capable of altering the measurement.

243 The electrochemical characteristics of crystalline epitaxial graphene grown on SiC were
 244 studied in (ref. 48) using a three-electrode electrochemical cell consisting of a graphene
 245 coated working electrode, an Ag/AgCl reference electrode with saturated KCl and a Pt wire
 246 as counter electrode. Graphene was anodized at 2 V in a pH 7 phosphate buffer solution to
 247 introduce oxygenated surface groups such as C=O and C–O–H. X-ray photoelectron
 248 spectroscopy (XPS) established that long anodization times (e.g. 500 s) led to the
 249 functionalization of the edges with hydroxyl and carboxylic groups. The hydroxyl and
 250 carboxylic groups increased the capacitive charging current, thus fastening the electron
 251 transfer kinetics. The sensitivity to pH was determined with the Mott-Schottky equation,
 252 which is commonly used to study the electrochemical interface between a semiconductor
 253 and an electrolyte solution⁴⁹. The total capacitance at the interface between graphene and
 254 an electrolyte solution is given by the sum of the reciprocals of the Helmholtz layer
 255 capacitance (C_H) and the space-charge capacitance of graphene (C_G). The typical
 256 assumption is that $C_H \gg C_G$ so that the Mott-Schottky equation becomes:

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$$\frac{1}{C_G^2} = \frac{2}{\epsilon\epsilon_0 eN} \left(V - V_{FB} - \frac{kT}{e} \right) \quad (1)$$

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259 where ε is the dielectric constant of graphene, ε_0 is the vacuum permittivity, e is the electron
260 charge, N is the donor density (holes or electrons), V is the applied potential, V_{FB} is the flat-
261 band potential (i.e. the potential at which the conduction and the valence bands of graphene
262 are flat), k is the Boltzmann constant, and T the temperature. When pH changed from 2 to
263 10, $\Delta V_{FB} = V_{FB}(pH = 10) - V_{FB}(pH = 2)$ for pristine graphene was much lower (0.1 V) than
264 for the anodized graphene (0.41 V). This result was reflected in a pH sensitivity of 12.5
265 mV/pH for pristine graphene and 51.3 mV/pH for anodized graphene. The better
266 performance of anodized graphene thus depended on the presence of the hydroxyl and
267 carboxylic groups.

268 The works described so far highlight that graphene-based FETs have ambipolar
269 characteristics, i.e. the charge carriers in the graphene channel were holes or electrons
270 depending on whether the V_G was smaller or greater than the V_{Dirac} . Nevertheless, Zuefuddin
271 *et al.* presented in 2013 an FET that was p-doped in the range $V_G \in [-30, 30]$ V⁵⁰. However,
272 this voltage range seems too wide and probably caused some defects in graphene. A
273 monolayer graphene was obtained by the decomposition of CH₄ in a CVD reactor onto a
274 Cu/SiO₂/Si substrate. After etching the copper with FeCl₃, graphene was transferred onto
275 SiO₂/Si and an array of FETs were fabricated using standard microlithography. The authors
276 suggested that the absence of a V_{Dirac} could depend on the p-doping from residuals left
277 during the transfer process, but the type of resist was not reported. The sensitivity to pH was
278 tested with different buffer solutions and there was an I_{DS} shift of 5% and 10% at pH 5.1 and
279 8.2, respectively. The repeatability and hysteresis were not reported.

280 Lin *et al.* used CVD on copper and nickel foils to grow graphene, which was then suspended
281 on an array of Cr/Pb sources and drains onto a SiO₂/Si substrate⁵¹. The channel width was
282 20 μm . The graphene grown onto nickel (Gr_{Ni}) was a continuous film with a low number of
283 defects and consisted of less than 10 layers, whereas the graphene grown on copper (Gr_{Cu})
284 was a quasi-continuous film consisting of a single-crystal monolayer. Since I_{DS} had an

285 exponential decay over time, the response was measured as $(I_{DS} - I_{DSin}) / I_{DSin}$, where I_{DSin}
 286 was the initial value. The Gr_{Ni} was tested at pH 7, 10, 11 and 13 and showed a linearly
 287 increasing response for increasing pH (4.75% at pH 7, 45.1% at pH 13, and 0.1 pH
 288 resolution). At $V_G = 0$ V and $V_{DS} = 0.1$ V, the I_{DSin} was not constant at different pH values
 289 (e.g. about 250 μ A at pH 7 and 1.5 mA at pH 13), thus indicating an irreversible adsorption
 290 of ions on graphene. The Gr_{Cu} was used at pH 7.35, 7.59 and 7.86 with sensitivities of
 291 34.5%, 47.6%, and 57.4%, respectively. The resolution was 0.01 pH. However, their paper
 292 does not report the number of measurements nor the error bars on plots.

293 The adsorption of ions onto a graphene surface and desorption have been studied via the
 294 effect on graphene conductivity. Kiani *et al.* proposed an analytical model to explain and
 295 predict the effect of electron exchange for a monolayer GNR⁵². The model used a typical
 296 ISFET configuration with gold contacts, SiO_2/Si substrate and an Ag/AgCl reference
 297 electrode. The conductance (G) of a GNR can be written as:

$$G = \frac{3q^2 \sqrt{3\pi a^3 t^3 k_B T}}{hl} [F_{-1/2}(\eta) + F_{-1/2}(-\eta)] \quad (2)$$

299
 300 where q is the electron charge, a is the graphene lattice constant (0.246 nm), $t = 2.7$ eV is
 301 the tight-binding energy for the nearest neighbour C-C atoms, k_B is the Boltzmann constant,
 302 T is the temperature, h is the Planck constant, l is the graphene channel length, $\eta =$
 303 $(E_F - E_g / k_B T)$ is the normalized Fermi energy (E_F is the Fermi level and E_g is the band gap
 304 energy), and $F_{-1/2}$ is Fermi-Dirac integral of order $-1/2$. The conductance at different pH
 305 (G_{pH}) was modelled as $G_{pH} = \frac{P}{pH} G$.

306 The parameter P is the pH sensing factor and, at $T = 25^\circ\text{C}$, can be calculated as $P =$
 307 $\alpha \ln(pH) + \beta$, where α and β are 2.7318 and 4.5044, respectively. This model showed a
 308 good agreement with experimental data from the authors (i.e. Kiani *et al.*) and (ref. 44).

309 Although the model was not sufficiently precise to fit the V_{Dirac} , the pH sensing factor can be
310 used to predict the conductance of the graphene channel when exposed to an electrolyte
311 solution.

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314 **Solution-gated ISFETs on a flexible substrate**

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316 The SiO₂/Si is widely used as substrate for the fabrication of graphene-based ISFETs.
317 However, an attempt has been made to produce these ISFETs on flexible substrates to
318 develop a technology capable of providing lightweight, bendable and/or stretchable,
319 potentially environment-friendly and cost effective devices. Maily-Giacchetti *et al.* compared
320 the performances of an SGFET fabricated on SiO₂/Si with a similar device on a 125 μm thick
321 poly(ethylene 2,6-naphthalenedicarboxylate) (PEN) substrate⁵³. Monolayer graphene films
322 were grown on copper foils by CVD and then transferred onto SiO₂/Si and PEN. Two SiO₂/Si
323 SGFETs were fabricated with and without moderate PMMA leftover in order to study the
324 effects of residues due to the microlithographic process. The leakage current for both SiO₂/Si
325 and PEN SGFETs was about 1 nA. The tests on pH in the range [4, 8] were performed at
326 $V_{\text{DS}} = 50$ mV and $V_{\text{G}} \in [-0.1, 0.5]$ V. The SiO₂/Si SGFETs with (SGFET-SiO₂-PMMA) and
327 without (SGFET-SiO₂) residues showed a positive V_{Dirac} shift at increasing pH with similar
328 sensitivities, i.e. 21 and 22 mV/pH respectively. The SGFET-SiO₂ was more p-doped than
329 the SGFET-SiO₂-PMMA, probably because of the annealing step at 500 °C in H₂/Ar to
330 remove PMMA. The PEN SGFET had a lower carrier mobility (300 cm² V⁻¹ s⁻¹) than the
331 SiO₂/Si SGFET (1250 cm² V⁻¹ s⁻¹) and this difference was ascribed to the higher scattering
332 in PEN than in SiO₂ because of the higher root mean square (RMS) surface roughness (~5–
333 10 nm for PEN and ~0.4–0.5 nm for SiO₂). Nevertheless, since the sensitivities were almost
334 equal, the authors concluded that the substrate does not influence the pH response. The

335 PEN SGFET showed a good reversibility over repeated pH measurement cycles
 336 (decreasing and increasing ramps), but hysteresis was present. Maily-Giacchetti *et al.* also
 337 studied the effect of the surface transfer doping due to the exchange of electrons between
 338 the adsorbed ions and graphene. The electron transfer reaction rate of graphene was
 339 reduced by fabricating an SGFETs on octadecyltrichlorosilane (OTS), as described in (ref.
 340 54). The OTS-SGET sensitivity was 18 mV/pH, comparable with those of SiO₂/Si and PEN
 341 SGFETs, thus suggesting a negligible surface transfer doping.

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343

344 **Solid gate and three dimensional FETs**

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346 SGFETs are characterized via a direct application of V_G to the electrolyte solution, which is
 347 the gate dielectric. However, as a consequence any variability of the solution can affect the
 348 sensor response. The fabrication of a solid gate onto the SiO₂ substrate with a dielectric
 349 layer sandwiched between this gate and the graphene channel (Fig. 2a) is another FET
 350 configuration that improves the integration and microfabrication, as no cumbersome gate
 351 electrode (compared with the transistor dimensions) is needed.

352 Zhu *et al.* proposed a solid gate FET with HfO₂ as dielectric for pH sensing⁵⁵. For a FET, the

353 transconductance $g_m = \frac{\partial I_{DS}}{\partial V_G}$ expresses the sensitivity and is directly proportional to the

354 capacitance of the dielectric (C_{ox}). Since HfO₂ is a high-dielectric constant material ($k \approx 25$),

355 a high capacitance (i.e. $C_{ox}(HfO_2) = k \frac{\epsilon_0}{t_{ox}} \approx 1.1 \mu\text{F}/\text{cm}^2$) is obtained for a thickness (t_{ox}) of

356 just 20 nm. The choice of a high- k gate dielectric allows the thickness of the dielectric and

357 the leakage current to be reduced, thus improving sensitivity. With a V_G from 0.6 to 1.6 V,

358 this solid gate FET had a sensitivity of about 57.5 mV/pH in the pH interval 5.3–9.3 and a

359 V_{Dirac} positively shifting at increasing pH. The FET was tested at $V_G = 0.75$ V for real-time
360 sensing, but no information on repeatability and hysteresis was reported.

361 A further possible improvement to the FET configuration is the adoption of a 3D graphene
362 channel to enhance the transistor performances and reduce the power consumption^{56,57}. A
363 graphene foam, i.e. a 3D network of single and double layer graphene, was grown on copper
364 and then transferred onto a glass substrate. Although the foam shrank after etching the
365 copper, the 3D structure was preserved. The Ti/Au source and drain had a contact
366 resistance of about $600 \Omega/\square$, a high value probably due to the non-uniform shrinkage of the
367 foam. The top side of the graphene foam was coated with a layer of 20 nm of HfO_2 that was
368 in contact with an electrolyte solution. The length and width of the transistor were 0.6 and 5
369 mm, respectively. With a $V_G \in [-1.5, 1.5]$ V, the leakage current was about 3 orders of
370 magnitude lower than I_{DS} . Figure 2b shows the 3D graphene FET. The pH response was
371 tested in the range [3, 9] and the sensitivity was 79 ± 7 mV/pH. The authors attributed this
372 super-Nernstian sensitivity to the combination of 3D graphene foam and HfO_2 , but this result
373 should be further investigated. The real-time monitoring was verified with a single
374 measurement at $V_S = -0.5$ V and $V_{\text{DS}} = 0.5$ V for pH decreasing from 9 to 3. Repeatability
375 and hysteresis were not reported.

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379 **Graphene quantum dots**

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381 Graphene quantum dots (GQDs) are low toxicity and high surface area particles with
382 diameters below 100 nm, typically between 3 and 20 nm. GQDs show quantum confinement
383 effects and a non-zero bandgap that can be tuned during preparation to modify the
384 photoluminescence (PL). The absorption spectra have a characteristic peak at about 230

385 nm and a typical PL quantum yield lower than approximately 30%. Their luminescence
386 properties can be improved by new fabrication methods (e.g. water-phase molecular fusion
387 and hydrothermal methods), doping or functionalization with organic, polymeric, inorganic,
388 or biological molecules⁵⁸⁻⁶³.

389 Although GQDs have only recently begun to show their potential for sensing applications,
390 they have already been used in imaging of living cells, as probes for single-stranded
391 deoxyribonucleic acid (ssDNA), detection of human immunoglobulin (IgG), adenosine
392 triphosphate (ATP), thiols, and cleavage of DNA. Furthermore, the chemical synthesis of
393 GQDs introduces oxygen-containing groups, e.g. carboxylic acid groups, and defects at the
394 edges and in the basal plane, which make GQDs a promising material for pH sensing⁶⁴⁻⁶⁸.

395 Huang et al. reported a wide PL emission range, from ultraviolet C to blue, for a GQD
396 diameter between 1 and 2 nm, whereas a PL red shift and a decreasing band gap were
397 observed for larger diameters. Furthermore, a blue shift was observed when pH moved from
398 basic to acidic, and this was ascribed to the moving up of the exciton energy level. The
399 authors suggested that the optical excitation could induce two kinds of excitons, i.e. within
400 the whole GQD or in the edge microstructure. At decreased pH values, the passivation of
401 the edge microstructure was responsible for the blue shift, whereas the wide emission
402 spectra for small diameter size depended on the strong quantum confinement due to the
403 excitons confined within the GQD⁶⁹.

404 Wu *et al.* synthesized nitrogen-doped GQDs (N-GQDs) using citric acid (CA) and
405 dicyandiamide (DCD) as the carbon and nitrogen sources, respectively⁷⁰. The N-GQDs were
406 1-2 graphene layers thick with an average diameter of 2.3 nm. The Fourier transform infrared
407 spectroscopy (FTIR) and the XPS showed the presence of $-\text{COOH}$, $-\text{OH}$ and $-\text{NH}_2$ groups.
408 In buffer solutions from pH 2 to 9 and at an excitation wavelength $\lambda_{\text{ex}} = 365$ nm, the
409 fluorescence intensity of N-GQDs was linearly proportional to pH and reversible during
410 multiple cycles between pH 2 and 9. The zeta potential versus pH showed that these N-

411 GQDs are positively charged for $\text{pH} < 2.7$ and negatively charged for higher values
412 (sensitivity ~ -4 mV/pH), which is a similar behaviour to that of amino acids. These N-GQDs
413 were also used to measure the pH of river water, rain and tap water and gave comparable
414 results with those of a commercial pH-meter. The biocompatibility was assessed with human
415 epidermoid cancer cell lines (Hep-2) at an N-GQDs concentration of $1 \text{ mg}\cdot\text{mL}^{-1}$ for 24 h. The
416 N-GQDs did not significantly alter the viability of cells and were permeable to cells
417 membranes with an increasing fluorescence intensity from pH 5.7 to 8.

418 One year later, in 2015, Shi *et al.* used 3,4-dihydroxy-L-phenylalanine(L-DOPA) as the
419 nitrogen source and the acronym N-OGQDs to stress that these GQDs were rich in oxygen
420 atoms⁷¹. Their material had an average diameter of 12.5 nm and contained 66.58 wt% C,
421 4.01 wt% N, 5.77 wt% H, and 23.64 wt% O. The N-OGQDs were stable in high-ionic strength
422 buffers (concentration of NaCl up to 1 M) and the PL intensity linearly increased in the pH
423 range [3, 8] ($\lambda_{\text{ex}} = 346$ nm). Given the fluorescence intensity in arbitrary units (a.u.), the
424 sensitivity was ≈ 80 a.u./pH. As for (ref. 61), the pH sensitivity can be ascribed to the
425 reactivity of the amide and carboxylic groups. Although the N-OGQDs seemed potentially
426 suitable for measuring pH in the physiological range, further tests are needed to evaluate
427 parameters such as repeatability and accuracy.

428 Another synthetic approach for GQDs entailed the electrolysis of a graphite rod in a 5 mL
429 dimethylsulfoxide (DMSO) solution with 0.01 M tetrabutylammonium perchlorate (TBAP)⁷².
430 Yuan *et al.* obtained GQDs that consisted of approximately 1-3 graphene layers with an
431 average diameter of about 10.6 nm. Sensitivity to pH was demonstrated in a wide range, i.e.
432 [1, 14], with a particular behaviour at $\text{pH} > 11$. From 1 to 11, the PL intensity peak shifted
433 from 522 to 575 nm due to the deprotonation of the functional groups, e.g. $-\text{COOH}$ and $-\text{OH}$,
434 which is in common with most previous pH sensors. However, from pH 12 to 14, the PL
435 had new absorption peaks at 520-560 nm and a strong red emission centred at about 625
436 nm. The emission at 625 nm was stable even when the excitation wavelengths were

437 changed from 360 to 560 nm. The FTIR and XPS spectra showed the presence of a lactone
438 structure that aroused by the tertiary alcohols reacting with nearby carboxylic acids at the
439 edges of GQDs. In strong basic pH conditions, Yuan *et al.* verified that the lactone turned to
440 quinone and caused the modification of the PL absorption spectra. In fact, when the GQDs
441 were reduced by NaBH_4 , which is known to eliminate lactone in graphene, there was a blue
442 fluorescence instead of a red emission at basic pH and the solution colour turned to yellow
443 instead of red at pH 13. From 1 to 13, the GQDs were pH-responsive from 10 up to 80°C
444 and had a reversible fluorescence response over six consecutive heating-cooling cycles in
445 this temperature range. The *in vivo* tests with human cervical cancer cells (HeLa) showed
446 visible colour changes with a culture pH increasing from 5 to 9.

447 Park *et al.* grafted blue-emitting poly(7-(4-(acryloyloxy)butoxy)coumarin)-b-poly(N-
448 isopropylacrylamide) (P7AC-b-PNIPAAm) to green-emitting 10 nm GQDs (block copolymer
449 integrated GQDs, bcp-GQDs)⁷³. At room temperature, the PL spectra of bcp-GQDs showed
450 two emission peaks following an excitement at 365 nm, a first peak at 410 nm corresponding
451 to the P7AC-b-PNIPAAm, and the other at 505 nm associated with the GQDs. The blue
452 emission peak at 410 nm did not change with pH, whereas the green emission peak changed
453 because of the protonation and deprotonation of the oxygen-based functional groups.
454 However, the intensity ratio I_{510}/I_{410} plotted versus pH from 1 to 11 did not allow several pH
455 values to be resolved, thus this promising idea should be further expanded with additional
456 work in order to be of practical application.

457 Song *et al.* fabricated GQDs via a modified graphite intercalated compounds (GICs)
458 method⁷⁴ and investigated the pH response⁷⁵. Two GQDs diameters were investigated, i.e.
459 GQD-A (2 ± 1 nm) and GQD-B (18 ± 2 nm), with an oxygen content of ~ 5 at% and ~ 8 at%,
460 respectively. The FTIR spectra showed that the oxygen content was mostly due to the
461 carboxyl groups and was lower than that of typical GQDs obtained from GO and rGO. In
462 particular, GQD-A had a lower content than GQD-B. The carboxylic acid peak was absent

463 in GQD-A and much lower in GQD-B. Raman spectra indicated that these GQDs had a few
464 defects and GQD-A had a stronger sp^2 hybridization than GQD-B. GQDs were tested with
465 excitation wavelengths from 250 to 390 nm and at pH 2, 7 and 12. For GQD-A, the maximum
466 PL intensity was at $\lambda_{\text{ex}} = 315$ nm at all pH values. The PL intensity decreased with increasing
467 pH and the spectra were blue-shifted, sharper and with higher intensities at pH 2 than at pH
468 7 and 12, probably because of the protonation effect at basic pH on the edges and defect
469 sites of GQD-A. Conversely, GQD-B had the lowest PL intensity at pH 2 and the maximum
470 at pH 7 and 12 for $\lambda_{\text{ex}} = 360$ nm, with an increasing trend from basic to acidic pH.

471 The nanosheets synthesized by Joseph *et al.* were very close to nanodots, with average
472 length and width of 135 and 137 nm, respectively, and a thickness of about 1-3 graphene
473 layers⁷⁶. These nanosheets were obtained by the aqueous exfoliation of graphite through
474 the interaction with an antibacterial protein, the lysozyme (LYS). Since the isoelectric point
475 (IEP) of LYS depends on the solution pH, the correlation between the LYS-nanosheets and
476 the zeta potential was studied at different pHs from 1 to 13. The LYS-nanosheets IEP was
477 about 8 with an initial pH of approximately 4. The zeta potential allowed the pH to be
478 discriminated only between pH 1 and 5. At pH values below the IEP, the amines in the LYS-
479 nanosheets led to a global positive charge that repelled the nanosheets from aggregations,
480 whereas above the IEP the predominant effect was ascribed to the carboxylate ions that led
481 to a global negative charge and a fine dispersion. On the other hand, around the IEP, the
482 LYS-nanosheets aggregated because of a charge depletion. Interestingly, when tested for
483 cytotoxicity with mouse embryonic fibroblasts (NIH-3T3) and three different cancer cells
484 lines, namely human colorectal cancer cells (HCT-116), HeLa and squamous carcinoma
485 cells (SCC-7), the LYS-nanosheets were less toxic for the NIH-3T3 than for the cancer cells.
486 Although the cytotoxicity mechanism needs further investigation, these nanosheets showed
487 promising properties for pH sensing.

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491 **Graphene oxide**

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493 Unlike graphene, GO is an electrical insulator due to its disrupted sp^2 bonding network,
494 though there are several methods to restore the electrical conductivity⁷⁷. Although the
495 precise chemical structure is still under investigation, the most widely accepted model was
496 developed by Lerf and Klinowski⁷⁶ and describes GO as graphitic platelets characterized by
497 epoxy and hydroxyl groups on the basal plane, and carboxyl groups on the edges. The large
498 number of highly polar groups makes GO highly hydrophilic and a good candidate for
499 measuring the pH of solutions. The pH dependence of GO in aqueous solutions has been
500 investigated and the change in GO hydrophilicity has generally been ascribed to the
501 protonation-deprotonation of the carboxyl groups⁷⁹⁻⁸¹. At high pH, the hydrophilicity is
502 enhanced and GO should behave like a surfactant, but since the surface tension is weak, it
503 dissolves in water like a regular salt. At low pH, the degree of deprotonation is low and GO
504 forms suspended aggregates with a sandwich-like structure GO-water-GO surrounded by
505 water molecules.

506 Wang *et al.* prepared a GO colloid using a modified Hummers method and studied the role
507 of pH, adjusted with HCl solution and aqueous ammonia, in the formation of a hydrogel^{82,83}.
508 The GO colloid aggregated into a cylindrical hydrogel at $\text{pH} \leq 5$ or ≥ 10 , whereas a black
509 dispersion was obtained otherwise. This result depended on i) the degree of dissociation of
510 carboxyl groups, whose charge state controlled the attraction-repulsion of GO sheets, ii) the
511 concentration of GO, and iii) the use of HCl and ammonia, which reduced the thickness of
512 the electrical double layer of the GO sheets.

513 With another modified version of the Hummers method, Qin *et al.* synthesized and dispersed
514 about 20-50 mg of GO in 1 mL of purified water to obtain a self-assembled GO hydrogel

515 without any pre-processing⁸⁴. The average size of the GO nanosheets was about 500 nm,
516 and the thickness was between 0.911 and 0.607 nm, respectively. The hydrogel was
517 obtained with a minimum GO concentration of 30 mg/mL. The gel formation was pH-
518 dependent and only occurred for acidic pH. The GO hydrogel was stable when the GO
519 concentration exceeded 5 wt%, probably because the $\pi - \pi$ stacking and the hydrophobic
520 interactions inhibited the pH-response. The ionization of the carboxyl groups, which
521 increases with pH and reduces the number of the hydrogen bonds, was also exploited in
522 hydrogels consisting of GO and poly(N-isopropylacrylamide), or GO and starch-based
523 superabsorbent nanocomposites (SANCS), i.e. magnetic iron oxide nanoparticles and
524 starch-g-poly(acrylic acid-o-acrylamide)^{85,86}.

525 Because of its insulator-like behaviour, the use of GO as pH sensor is generally associated
526 with optical analysis. Lv *et al.* ultra-sonicated 2 mg/mL GO for 2 h to obtain GO hydrosols
527 whose pH was adjusted from 2 to 11 with HCl or KOH aqueous solution⁸⁷. Outside the pH
528 range [2, 11], the hydrosol became unstable. After the centrifugation to remove the residual
529 nanosheets, the GO hydrosols were heated in a water-thermal bath at about 85 °C for 30-
530 45 min and formed thin membranes at the liquid/air interface. These GO membranes
531 (GOMs) had a thickness of between 1.5 and 2 μm at pH 11 and 2, respectively. According
532 to the ultraviolet-visible (UV-vis) spectra, the highest optical transmittance at 600 nm was
533 about 30% at pH 2, and non-linearly decreased to about 1% at pH 11 (Fig. 3a). Lv *et al.*
534 explained that this result was due to the deoxygenation of the GOMs in basic conditions,
535 which led to a partial reduction of GO. The transmittance increased with increasing
536 wavelength and was 65% at 1000 nm and pH 2. The transmittance of the GOMs at pH 2, 7
537 and 11 was also determined in the range [200, 3300] nm. Perhaps because of the presence
538 of water molecules, the GOMs transmittances showed a non-linear dependence after 1300
539 nm, which can be thus assumed as the wavelength limit to use these GOMs as pH sensors.

540 Yan *et al.* proposed an optical pH sensor consisting of a film made up of polyethylenimine
541 (PEI) functionalized lanthanide-doped (NaYF₄:Yb,Er) upconversion nanoparticles (PEI-
542 UCNPs) and GO⁸⁸. GO is a pH-dependent quencher for the luminescent PEI-UCNPs, and
543 the fluorescent intensity can be used to sense pH. An amount of 0.5 mg/mL of PEI-UCNPs
544 at pH 5 was mixed with 1 mg/mL GO and, after vacuum filtration and washing with water, a
545 film was obtained leaving this dispersion to dry in air for two weeks. The GO-PEI-UCNPs
546 film was dipped in buffer solutions and excited with a 980 nm laser. The fluorescence
547 intensity at 540 nm linearly decreased (maximum 35%) when the pH changed in the
548 physiological range from 5 to 8, with a response time of about 60 s for unitary changes of
549 pH. The repeatability, stability and reversibility were demonstrated by cycling the film
550 between pH 6 and 8 for five hours. This sensor was also tested with mice urine, which was
551 diluted to obtain different pH conditions. The response was almost linear, with a maximum
552 intensity shift of 55% between pH 4.95 and 8.05 (Fig. 3b). The GO-PEI-UCNPs film was
553 found to be highly biocompatible *in vitro* when in contact with cultures of RAW264.7, MDA
554 and MC3T3-E1 cell lines.

555 Chen *et al.* monitored the vis-near infrared (vis-NIR) fluorescence of GO to determine the
556 changes in the extracellular pH during growth and metabolism of normal and cancer cells⁸⁹.
557 In buffer solutions, the fluorescence intensity at 650 nm of the single-layer GO nanosheets
558 had a reversible sigmoidal response when the pH changed from 2 to 12 (Fig. 3c). The pH
559 changes were also monitored in cultures of chronic granulocytic leukaemia (CGL) cancer
560 cell (32D-BA) and normal mouse cells (32Dc-13). This test showed that the GO optical
561 nanosheets were in linear correlation with a commercial pH meter ($R^2 = 0.99$) between pH
562 6.6 and 7.7.

563 In another work, PAA and poly(2-vinylpyridine) (P2VP) were grafted onto the surfaces of
564 CdS/ZnS (blue emission at 440 nm) and CdSe/ZnS (orange emission at 580 nm) QDs,
565 respectively. These two compounds were anchored to GO by $\pi - \pi$ stacking interactions

566 between the pyrene groups of PAA and P2VP and the basal plane of GO. The GO-QDs (1
567 mg/mL) were characterized in aqueous media at different pH conditions with a λ_{ex} of 365
568 nm. The PL intensity of the blue peak was almost stable from pH 1 to 4, and increased from
569 pH 4 to 7. On the other hand, the PL intensity of the orange peak decreased from pH 1 to 5,
570 and saturated after pH 5. This effect could be explained by the fact that for $\text{pH} < \text{pKa} = 3$ the
571 P2VP chains swelled (P2VP-QDs size increased from ~45 to ~85 nm), whereas the PAA
572 chains swelled for $\text{pH} > \text{pKa} = 4.5$ (PAA-QDs size increased from ~30 to ~70 nm). The
573 swelling reduced the GO quenching and favoured the blue or the orange emission
574 depending on the pH value. The ratio of the two intensities, I_{580}/I_{440} , plotted versus pH from
575 1 to 7 showed a non-linear relationship that could be linearly approximated between pH 1
576 and 5 (sensitivity $\sim -0.56/\text{pH}$, Fig. 3d). These GO-QDs were also reversible when cyclically
577 exposed to acid and basic pH⁹⁰.

578 A pH sensing material was obtained by combining DNA probes and GO in a work by Luo *et*
579 *al.*⁹¹. This sensor was fabricated by incubating DNA probes marked with the fluorescent dye
580 tetramethylrhodamine (TAMRA, 100 nM) in 20 μL phosphate buffer at different pH values
581 for 5 minutes, then adding ultra-pure water (68 μL) and GO (12 μL , concentration 100
582 $\mu\text{g}/\text{mL}$). Two DNA probes were designed to have different contents of the protein Tat, 50%
583 (probe 1) and 80% (probe 2). Tat allowed the DNA conformation to change at different pH
584 values, whereas the fluorescence signal of TAMRA was independent of pH. At basic pH,
585 the DNA probes had a hairpin structure with single stranded tails and were absorbed onto
586 the GO surface, thus the fluorescence was quenched. At acidic pH, the DNA probes
587 assumed a triplex structure, which is more rigid than the single stranded tail conformation.
588 In this case, the probes were not absorbed, and the fluorescence was not quenched. The
589 different Tat contents made the probes change the conformation at different pHs, thus
590 enabling the different pH values to be sensed. For $\text{pH} < 5$, probe 1 had a weak fluorescence
591 intensity and increased from pH 5 to 7. For $\text{pH} > 7$, the intensity saturated. Probe 2 had a

592 similar behaviour with a dynamic range between pH 6 and 8. Therefore, a combination of
593 probes 1 and 2 could be used to measure pH from 5 to 8.

594 Melai *et al.* presented an electrochemical GO pH sensor with a gold three-electrode screen
595 printed set-up. The working electrode (WE) was coated by a 400 nm GO layer and the
596 reference electrode (RE) was Ag/AgCl (the counter electrode was short-circuited to RE)⁹².
597 The open-circuit circuit potential between WE and RE was linearly related to pH (range [4,
598 10]) in buffer solutions, with a sensitivity of about 32 mV/pH. This sensor was intended to
599 monitor wound pH, and measured values only differed less than 0.1 pH unit from those from
600 a commercial pH meter over a 4-day test in human exudate, i.e. a fluid mainly produced in
601 the inflammatory and proliferative phases of a wound⁹³.

602 Salvo *et al.* expanded the previous work and used a GO dispersion (4 mg/mL) to fabricate
603 a pH responsive film in the range [5, 9]. The resulting sensor (sensitivity ~43 mV/pH,
604 repeatability 0.2 pH unit and high linearity $R^2 = 0.99$) performed comparably to a commercial
605 pH meter (error = 0.14 ± 0.09 pH units) when tested in human plasma over one month⁹⁴.

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609 **Reduced graphene oxide**

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611 Like for GO, the literature reports several attempts to produce pH-sensitive rGO-based
612 dispersions. Liu *et al.* proposed pyrene-terminated poly(2-N-(dimethyl amino ethyl acrylate)
613 (PDMAEA) and PAA for polymer-rGO composites, but the pH-dependence of rGO was
614 negligible⁹⁵. In another paper, after the synthesis of GO by a modified Hummers method,
615 Yang *et al.* added 10 mg of lysozyme from hen egg white to 20 mL of a GO solution before
616 reduction with 1 mL of hydrazine hydrate. After heating, ultra-sonication and centrifugation,
617 a lysozyme-rGO (lys-rGO) solution was obtained. The lysozymes adsorbed on GO, probably

618 due to π - π stacking and electrostatic interactions. They then acted as a dispersant, leading
619 to a low folding degree and flat lys-rGO with a higher carbon-oxygen (C/O) atomic ratio than
620 that of bare rGO. Furthermore, the lysozymes promoted the reduction of the oxygen-based
621 groups of GO, thus enhancing the restoration of the sp^2 hybridization, and stabilized the rGO
622 dispersion. The zeta-potential increased in absolute value when pH increased from 2.36 to
623 12.5, and were assumed to be linear between pH 4 and 10 (Fig. 4a)⁹⁶.

624 Liu *et al.* added chitosan to a GO solution before the reduction with hydrazine⁹⁷. The zeta
625 potential was higher than 30 mV for $2 < \text{pH} < 5$, probably because of the strong chitosan
626 protonation. After pH 5, the zeta potential rapidly decreased to about 9 mV at pH 6 and was
627 negative for $\text{pH} \geq 7$ (Fig. 4b). This effect was probably due to the increased ionization of the
628 carboxylic groups in rGO and the decreased chitosan protonation. These effects led to the
629 aggregation of rGO at pH values higher than the pKa of chitosan (6.5) because the
630 electrostatic repulsive forces became too weak. The aggregation of rGO was highly
631 reversible when tested in repetitive cycles between pH 4 (dispersion) and 7 (aggregation).
632 The same behaviour was observed between pH 7 (aggregation) and 10 (dispersion). Ren
633 *et al.* described a polyacrylamide (PAM)-rGO composite that could be dispersed for $\text{pH} \geq$
634 4⁹⁸. Although the PAM-rGO composite showed a pH dependence when characterized by
635 UV-vis, this optical analysis does not seem ready for pH sensing because the discrimination
636 between different pH values still needs improving.

637 Yang *et al.* treated GO with ozone before and after a thermal reduction process to obtain an
638 ozonized rGO quantum dots solution (O-rGO-QD)⁹⁹. After the thermal reduction, the rGO
639 sheets were treated with ozone in water solution at pH 2 (O-rGO1) and 13 (O-rGO2), and
640 hydrothermally treated at $\text{pH} < 1$. Four other types of rGO sheets (O-rGO3 to O-rGO6) were
641 obtained after ozonation at pH 2, 7, 10 and 13, and a hydrothermal treatment at pH 13. After
642 drying, the O-rGOs were filtered through a microporous membrane to obtain a solution of
643 O-rGO quantum dots (O-rGO-QD_x, $x = 1..6$, diameter 2-5 nm for $x = 1$ and 2, and 3-5 nm

644 for $x = 3.6$). The re-oxidation of GO through ozone formed carboxylic and ketone groups,
645 i.e. reactive sites for thermal reduction. These reactive sites were also introduced with the
646 ozone treatment after the reduction. The percentage of reactive sites depended on the pH
647 of the hydrothermal treatment, which increased the oxygen percentage from about 16% for
648 O-rGO-QD1 and O-rGO-QD 2 to a range of about [17, 27] % for O-rGO-QD3 up to O-rGO-
649 QD6. With an excitation wavelength of 254 nm, the fluorescence emission peak shifted
650 towards shorter wavelengths when pH increased from 2 to 13 during ozonation. Yang *et al.*
651 hypothesized that this phenomenon could be explained by the reaction of rGO with one of
652 the two forms of ozone in acid–base aqueous solution, i.e. dissolved ozone or HO \cdot radicals.
653 In acidic solution, the dissolved ozone is more reactive than the HO \cdot radicals, whereas the
654 opposite happens at basic pH. The O-rGO-QD1 fluorescence emission peak at 404 nm
655 reversibly increased for pH from 1 to 13 pH, with a negligible change in shape of the intensity
656 curve and a strong variation for pH > 7. The authors suggested several theories that could
657 explain this result, such as the triplet ground state¹⁰⁰ and the islets theories¹⁰¹.

658 Srinives *et al.* fabricated an rGO pH sensor using L-ascorbic acid (L-AA) as the reducing
659 agent¹⁰². An rGO layer of unknown thickness and size bridging two electrodes (the
660 electrodes distance is not reported) was used to fabricate a chemiresistive sensor. The
661 normalized resistance ($\Delta R/R_0$) to the rGO resistance in air ($R_0 \sim 6.7$ K Ω) decreased for
662 unitary changes of pH from 3 to 8 with a sensitivity of 0.5 pH⁻¹, roughly. However, this study
663 does not seem mature enough for pH sensing as stability, repeatability, cycling tests and
664 large variations at low pH should be further characterized.

665 In 2013, Sohn *et al.* published the first paper on a solution-gated rGO FET for pH
666 detection¹⁰³. The rGO FET had a SiO $_2$ /Si substrate and the SiO $_2$ (100 nm thick) was exposed
667 to a 3% 3-aminopropyltriethoxysilane (APTES) aqueous solution. The APTES positively
668 charged SiO $_2$ with amino functional groups, so that the aggregation of GO nanosheets was
669 prevented when a GO solution was dropped on the substrate (nanosheet thickness ~ 1 nm,

670 diameter 0.5–1.5 mm). The rGO channel (resistance ~10–20 K Ω) between source and drain
671 (length 400 μm , ratio width/length = 20) was obtained by reducing GO with hydrazine. The
672 V_G was applied through an Ag/AgCl electrode in the range [-0.2, 0.2] V and constant $V_{DS} =$
673 0.1 V. The device had a $V_{Dirac} \cong 0 \pm 0.01$ V at pH 7 and low rGO carrier mobility (0.5
674 $\text{cm}^2/(\text{V}\cdot\text{s})$) due to the residual –OH and –COOH functional groups on the rGO surface after
675 reduction. The rGO FET was tested from pH 6 to 9 with unitary increments. The response
676 was almost linear ($R^2 \cong 0.98$) with a sensitivity, i.e. $\Delta V_{Dirac}/\Delta\text{pH}$, of about 29 mV/pH. The
677 reversibility seems good from the reported plot, but no numerical error was given. The
678 repeatability error increased for increasing pH, with a maximum of about 20 mV at pH 9, i.e.
679 almost one pH unit. The pH values from 9 to 6.11 were correctly discriminated when the
680 rGO FET was tested during real-time measurements at constant $V_G = -0.2$ V. Unlike
681 graphene, the rGO has oxygen functional groups in its structure, thus it is reasonable to
682 assume that the modulation of the reduction process could improve the FET performances.
683 Li *et al.* started with a SiO_2/Si substrate anisotropically etched to obtain reverse pyramids
684 (35 μm deep, pitch distances 18, 35, 70, 105 and 140 μm) in Si (Fig. 4c)^{104,105}. After spraying
685 a commercial rGO solution onto the substrate and heating at 120 $^\circ\text{C}$, the rGO was annealed
686 in nitrogen at 300 $^\circ\text{C}$ for 1h and treated with oxygen plasma for 1 min. The sensing window
687 was 4 x 4 mm^2 per sample. Transmission electron microscopy (TEM) and XPS analyses
688 showed that the oxygen-plasma-treated (OPT)-rGO FET had more oxygen functional
689 groups than the rGO FET. In terms of pH sensitivity, at $V_G \in [0, 4]$ V and $V_{DS} = 0.2$ V, the
690 OPT-rGO FET (sensitivity ~53 mV/pH) performed better than the rGO FET on planar Si (45
691 mV/pH), whereas the OPT-rGO FET with the reverse-pyramid structure (RP-OPT-rGO FET)
692 was even better (57.5 mV/pH for a pitch/depth ratio of 0.5) with high linearity ($R^2 = 0.996$)
693 because of the increased sensing surface. The optimal power for oxygen plasma treatment
694 was 20 W, since a decrease in sensitivity was observed at higher values. Hysteresis, defined
695 as the voltage offset between the beginning and the end of the test, was calculated for the

696 pH loop sequence 7, 3, 7, 11 and 7. For rGO, OPT-rGO and RP-OPT-rGO FETs, the offsets
697 were about 19, 13, and 11 mV, respectively. Therefore, the RP-OPT-rGO FET outperformed
698 the other configurations.

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701 **Conclusions and outlook**

702

703 Developing a graphene-based pH sensor is challenging. Although graphene-based
704 materials have pH-dependent electrochemical properties, current pH sensors are not
705 sufficiently advanced for new products to enter the market.

706 In this review, the field effect transistor was the most used pH-sensing architecture. Table 2
707 summarizes the efforts reported in the literature to create a graphene-based pH sensor.
708 Most FETs adopt graphene as the conduction channel, whereas there are only two
709 examples of rGO FETs. The sensitivity of these devices ranges from about 6 to 99 mV/pH
710 and such a wide spectrum suggests that researchers are still looking for an optimum
711 solution. Pristine graphene is scarcely sensitive to pH, but defects such as oxygenated
712 functional groups can modify its chemical structure and turn it into a pH-sensitive material
713 that can rival other existing pH sensors.

714 However, a standard fabrication process is not yet available and small differences can lead
715 to large variations. The control of defects is of primary importance to obtain reliable pH
716 sensors, especially the amount of -COOH groups at the graphene edges. It has been
717 demonstrated that oxygen plasma treatment or anodization can increase the number of
718 reactive sites to H^+ and OH^- ions. Another improvement could be the increase in the pH
719 sensitive area, as some solutions with suspended and 3D graphene have demonstrated.

720 These approaches can pave the way towards a standardized fabrication technology, but
721 they are only one side of the whole picture. Unfortunately, the pH response can be affected

722 by impurities and residual resist such as silanol and silylamine groups that modify the net
723 charge of the substrate surface, typically SiO_2 on Si. This problem could be solved by
724 improving the manufacturing techniques, but the effect of interfering ions such as Na^+ , Cl^-
725 and K^+ could still be a serious limiting factor.

726 This review has presented proof-of-concept pH sensors usually tested in reference buffer
727 solutions. However, some works showed that the pH response changes when electrolytes
728 are added to the buffer solutions. This could limit the applicability of these sensors in real
729 case studies. Quantum dots based on graphene, GO and rGO are an emerging alternative
730 to FETs to bridge the performance gap between the FETs and pH sensing (Table 3). For
731 example, the GQDs described in ref. 63 were successfully tested in river, rain and tap water,
732 and passed the biocompatibility tests. However, GQDs are still confined to lab research
733 because of limitations, such as the difficulty to achieve large scale production and high
734 quantum yield, and to understand correctly the quantum confinement and the doping mode
735 (e.g. lattice and edge modes)^{69,106,107}.

736 The future of graphene-based pH sensors will probably be decided by the ability of
737 researchers to produce effective results with FETs and GQDs. Although they can be
738 combined with other pH-sensitive materials, graphene, GO and rGO are likely to have a
739 considerable impact on pH sensing as stand-alone pH sensitive materials and this entails a
740 deeper understanding of the chemical interactions with hydrogen ions.

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751

752 Author contributions

753 P.S. conceived and wrote the paper. P.S., B.M., N.C. and C.P. were responsible of the
754 graphene and graphene oxide sections. P.S., A.K. and F.B. were responsible of the reduced
755 graphene oxide section. M.G.T. and R.F. were responsible of pH chemistry throughout the
756 paper. F.D.F. supervised and edited the paper.

757

758 Competing financial interests

759 The authors declare no competing financial interests.

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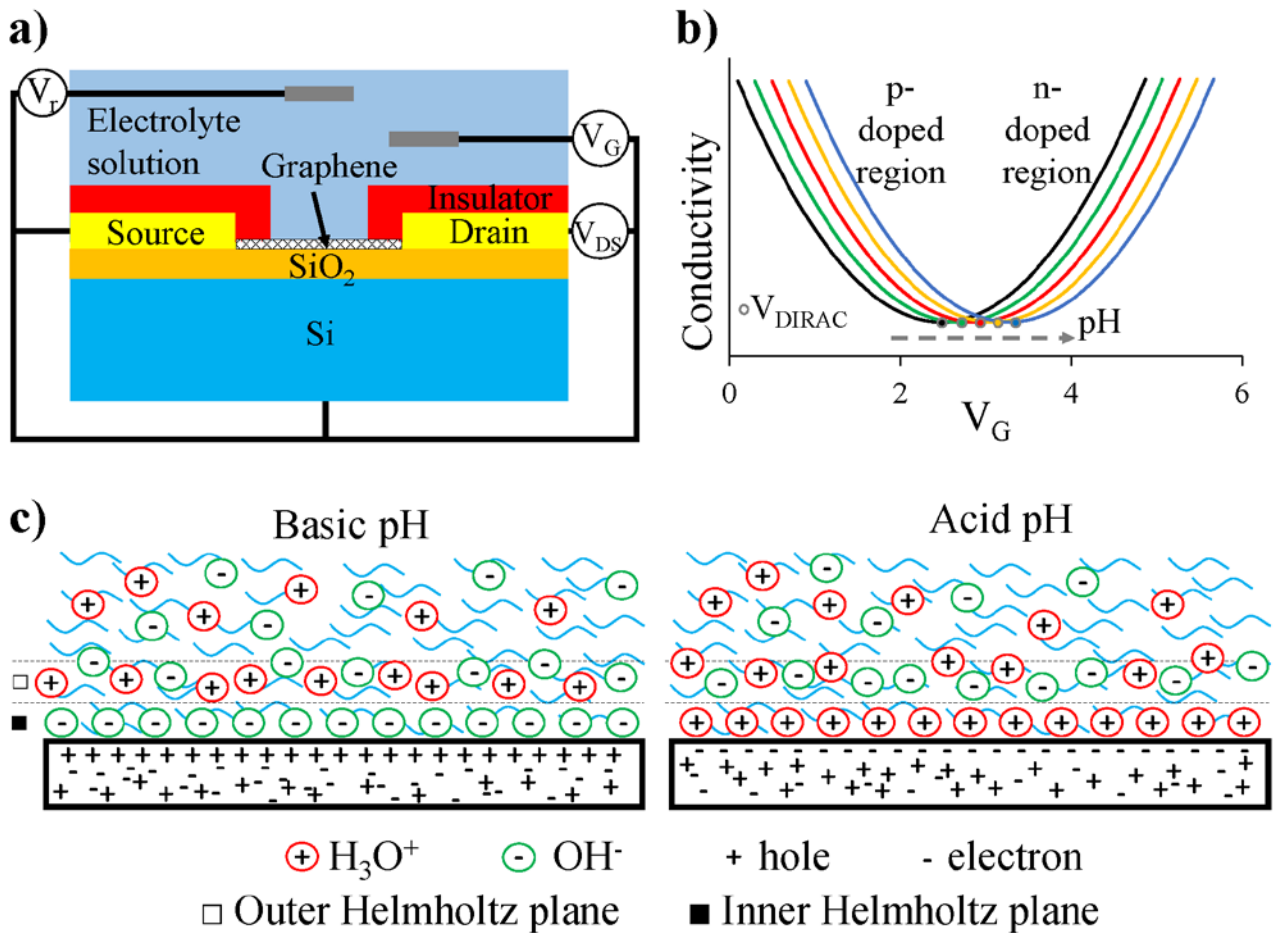
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1023 Figures

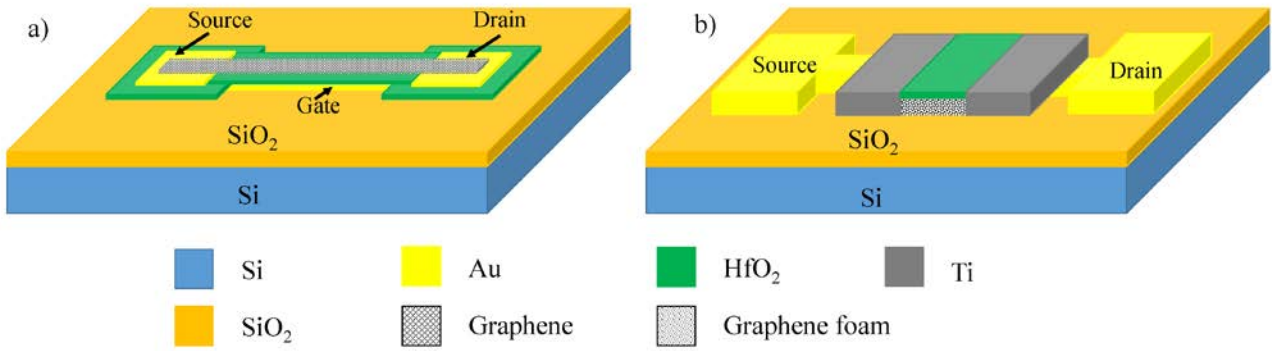
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1026 Figure 1. a) Three-electrode configuration of a pH-measuring FET. Although the most
 1027 common configuration is with V_G applied through a Ag/AgCl reference electrode and no
 1028 counter electrode, this ISFET provides the most accurate measurement; b) example of the
 1029 V_{Dirac} shift when pH increases. The area to the left of V_{Dirac} is associated with an excess of
 1030 holes in the graphene channel, and with an excess of electrons to the right. The
 1031 determination of the V_{Dirac} allows the measurement of pH; c) Distribution of ions and donors
 1032 (holes or electrons) at the graphene-electrolyte solution at basic and acid pH.

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1035 Figure 2. a) Field effect transistor with a solid gate under a HfO₂ layer; b) Three dimensional
 1036 field effect transistor where a graphene foam coated with a 20 nm HfO₂ layer is the pH-
 1037 sensitive material (modified from refs. 56 and 57).

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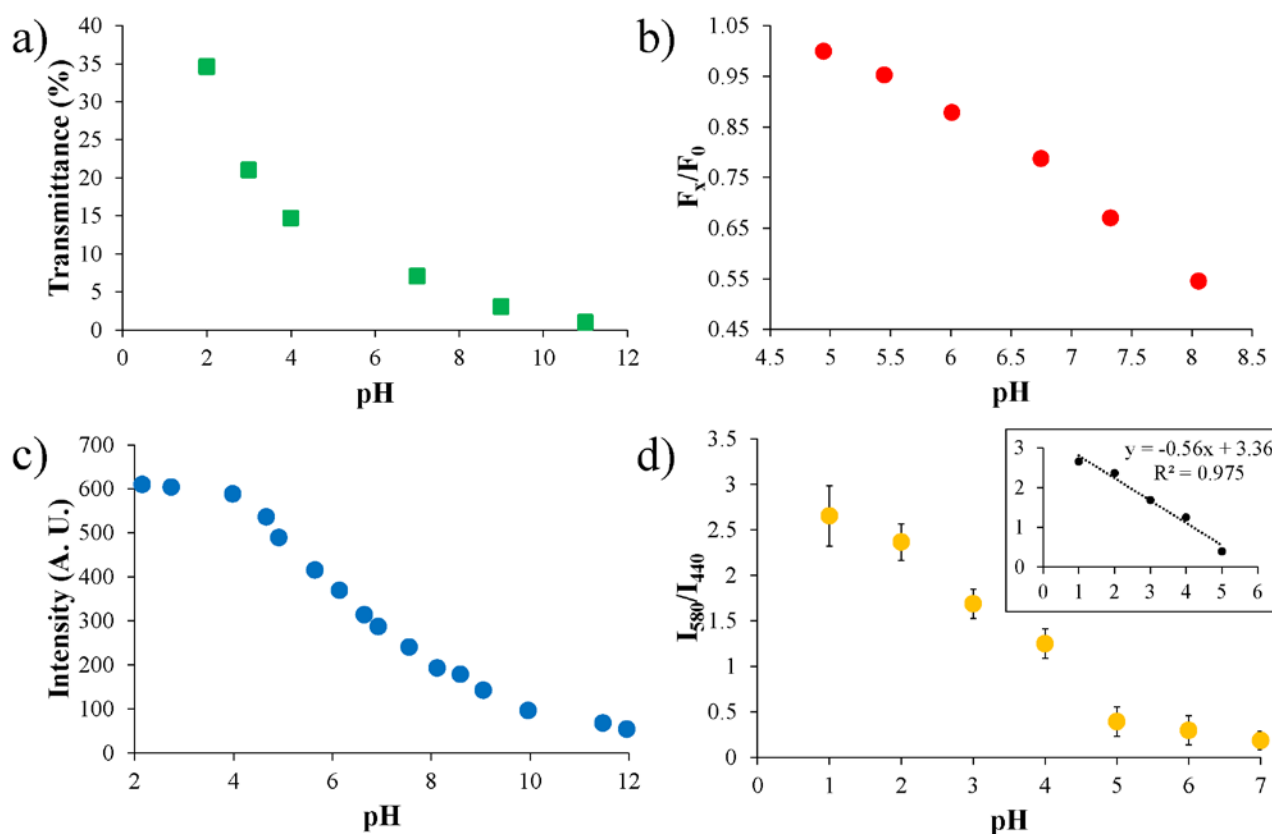
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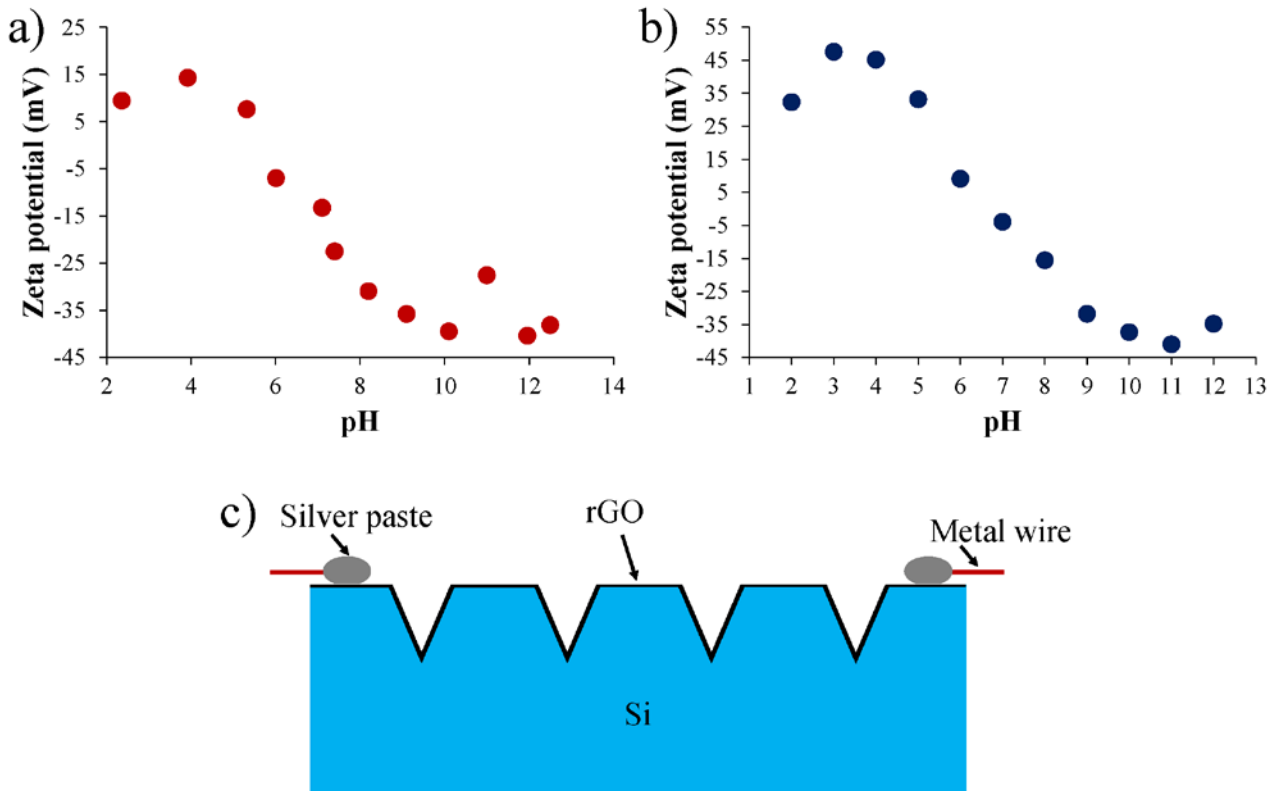


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1045 Figure 3. a) Optical transmittance of the GOMs at 600 nm. The decrease in transmittance
 1046 depended on the deoxygenation and partial reduction of the GOMs in basic conditions
 1047 (modified from ref. 87); b) Response of the GO-PEI-UCNPs in solutions of diluted mice urine.
 1048 F_0 is the fluorescence intensity at 540 nm at pH 5, whereas F_x is the fluorescence intensity
 1049 at the pH under test (modified from ref. 88); c) fluorescence intensity of the GO single-layer
 1050 nanosheets at λ_{ex} of 650 nm. The intensity can be linearly approximated in the pH
 1051 physiological range [4, 8] (modified from ref. 89); d) response of the PAA-P2VP-GO-QDs
 1052 reported as the ratio between the orange (I_{580}) and the blue (I_{440}) emission intensities versus
 1053 pH. Inset: linear approximation between pH 1 and 5 (modified from ref. 90).

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1057 Figure 4. a) pH dependence of a lysozyme-rGO solution. The lysozyme improved the
 1058 restoration of the sp^2 facilitating the reduction of the oxygen-based groups of GO (modified
 1059 from ref. 96); b) pH dependence of a chitosan-rGO solution. For $\text{pH} > \text{pKa} = 6.5$ of chitosan,
 1060 the rGO started aggregating (modified from ref. 97); c) the reverse-pyramid rGO-FET, which
 1061 had a higher pH sensitivity than the rGO-FET because of the increased sensitive area. An
 1062 oxygen plasma treatment further improved the sensitivity. (modified from ref. 104).

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1073 Table 1. Concentrations and ionic strengths of the solutions used to determine the effects
1074 of different electrolytes. The buffers were obtained in 0.05 M phosphate solutions, and 0.05
1075 M potassium was present in chloride and sodium solutions (adapted from ref. 42).

Electrolyte	pH	Concentration (M)	Ionic strength (M)
Na ⁺	6	0.0056	0.1224
	7	0.0279	0.2116
	8	0.0463	0.2852
K ⁺	6	0.0556	0.1224
	7	0.0779	0.2116
	8	0.0963	0.2852
Cl ⁻	6	0.0444	0.2112
	7	0.0221	0.2558
	8	0.0037	0.2926

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1082 Table 2. Characteristics of the graphene and rGO field effect transistors for measuring pH.

FET Configuration	Substrate	V_G (V)	V_{DS} (V)	Sensitivity (mV/pH)	pH range	Ref.
Solution-gated	6H-SiC	[-1, 1]	-1	99	[2, 12]	36
Gate-free	SiO ₂ /Si	Not applicable	Not reported	2.13 K Ω /pH	[4, 10]	37
Gate-free	Paper	Not applicable	[-1, 1]	30.8 Ω /pH	[1 10.5]	38
Solution-gated	SiO ₂ /Si	[-0.05, 0.05]	Not reported	30	[4, 8.2]	39
Solution-gated	6H-SiC	[-1.2, 0.3]	-0.05	19	[3, 12]	40
Solution-gated, suspended graphene	SiO ₂ /Si	[-0.05, 0.05]	Not reported	17	[6, 9]	41
Solution-gated	SiO ₂ /Si	[0, 1]	0.05	-78, -38 and -7 for 0.05 M Na ⁺ , Cl ⁻ and K ⁺ solutions, respectively. +69 for reference buffers	[6, 8]	42
Solution-gated	SiO ₂ /Si	[0.1, 0.8]	[0.01, 0.05]	6	[5, 10]	44
Solution-gated, graphene mesh	SiO ₂ /Si	[-0.1, 0.6]	0.05	90 (single use) 7 (after 3 cycles)	[6.55, 8.25]	45
Solution-gated, graphene nanoribbons	SiO ₂ /Si	[0.07, 0.32]	0.01	23.6	[6, 8]	46
Solution-gated, suspended graphene nanoribbons	SiO ₂ /Si	[-1.8, 1.8]	1	25	[5, 9]	47

Solution-gated, anodized graphene	SiC		Not reported	51.3	2 and 10	48
Solution-gated	SiO ₂ /Si, PEN	[-0.1, 0.5]	50	22 (both substrates)	[4, 8]	53
Solid gate, HfO ₂ dielectric	SiO ₂ /Si	[0.6, 1.6]	Not reported	57.5	[5.3, 9.3]	55
Solid gate, 3D graphene	SiO ₂ /Si	[-1.5, 1.5]	0.5	79 ± 7	[3, 9]	56, 57
Solution-gated, APTES treatment, rGO	SiO ₂ /Si	[-0.2, 0.2]	0.1	29	[6, 9]	103
Solution-gated, reverse-pyramid, oxygen plasma treated, rGO	Si	[1.3, 2.4]	0.2	57	[1, 13]	104, 105

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1094 Table 3. Characteristics of the graphene-base quantum dots.

Starting Material	Size (diameter, nm)	Excitation wavelength (λ_{ex} , nm)	Sensitivity	pH range	Ref.
Nitrogen-doped graphene	2.3	365	-4 (mV/pH)	[2, 9]	70
Nitrogen-doped (from L-DOPA) graphene	12.5	346	~80 (a.u. of fluorescence intensity)/pH	[3, 8]	71
Graphene (in 5 ml DMSO solution which contained 0.01 M TBAP)	10.6	365	~5 nm/pH	[1, 14]	72
P7AC-b-PNIPAAm-grafted graphene	10	365	Not reported	[1, 11]	73
Graphite powder	2 and 18	250 to 390	Not reported	[2, 12]	75
PAA-CdS/ZnS and P2VP-CdSe/ZnS nanodots anchored to graphene oxide	PAA-CdS/ZnS = ~85 at pH 7 P2VP-CdSe/ZnS = ~70 nm at pH 7	365	~-0.56/pH	[1, 7]	90
Ozononized reduced graphene oxide	2-5	254	Not reported	[1, 13]	99

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