

1 **Development of Low-Cost colourimetric and pH sensors based**
2 **on PMMA@Cyanine Polymers.**

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17

18 **Abstract**

19 Cyanine derivatives **1-3** were successfully characterized and their sensorial ability
20 studied against Zn²⁺, Co²⁺, Cd²⁺, Ni²⁺, Cu²⁺, Hg²⁺ and Ag⁺ metal ions. All dyes revealed
21 a colourimetric selectivity towards Cu²⁺ metal ions, with the formation of mononuclear
22 species. Cyanine **2** can detect and quantify the lowest concentrations of Cu²⁺ in aqueous
23 media, 0.3 and 0.6 µM. With the aim of exploring the optical properties of these dyes,
24 solid-supported devices were designed by the synthesis of polymethylmethacrylate
25 (PMMA) polymers doped with dyes **1**, **2**, and **3** and their copper complexes. All
26 PMMA_doped polymers were tested as temperature and low-pH sensors. PMMA_1 and

27 PMMA_1@Cu²⁺ showed to be reversible fluorimetric thermoresponsive polymers, while
28 PMMA_2, PMMA_3, PMMA_2@Cu²⁺ and PMMA_3@Cu²⁺ irreversible colourimetric and
29 fluorimetric thermoresponsive polymers. From all acids tested, HCl, HBr, HClO₄, H₃PO₄,
30 HCOOH and CH₃SO₃H, the best results were obtained for PMMA_3 and PMMA_3@Cu²⁺
31 with HCl. After activation in 12 M HCl, both polymers were capable of sensing 8 M, 10
32 M, 11 M and 12 M HCl: their color changed from green to yellow and they showed an
33 increase in their emission maxima at 805 nm in seconds. The capability to reversibly
34 sense higher acid concentrations in seconds, after activation, for more than 10 cycles,
35 make PMMA_3 and PMMA_3@Cu²⁺ good candidates for superacid sensing, allowing
36 for a real-time follow up of contaminated effluents, or their incorporation in quality control
37 in industrial processes.

38 **Keywords:** Colourimetric, Fluorimetric, metal ions, Temperature, Cyanine dyes, Low-
39 cost Polymers, Low-pH sensor polymers.

40 1. Introduction

41 Recognition and detection of analytes with environmental and biological impact is a hot
42 topic in chemistry and biology [1], [2]. Among all available analytical methods, optical
43 sensors highly sensitive and selective to analytes *via* colourimetric changes are the best
44 methods of detection because they are affordable and non-destructive [1], [3], [4].

45 Cyanine dyes have remarkable photophysical and photochemical properties, with strong
46 fluorescence and absorption in the near-infrared and visible region [1]. Due to their
47 outstanding biocompatibility and low toxicity in living systems, they are widely used in
48 biochemistry, biomedicine, imaging and as analytical reagents over a wide pH range [5],
49 [6], [7], [8], [9], [10].

50 Classical cyanine dyes contain two nitrogen-containing heterocycles, one of which is
51 positively charged. Both heterocycles are conjugated by a chain of carbon atoms:
52 according to the length of the chain, they can be classified as monomethine (Cy1),

53 trimethine (Cy3), pentamethine (Cy5) and heptamethine (Cy7) [1]. The absorption and
54 emission spectra of cyanines can be tuned by varying the length of this conjugated
55 polymethine chain; Cy3 has a greenish-yellow fluorescence, Cy5 and Cy7 are
56 fluorescent in the red and near-infrared region [1].

57 Chromophores with near-infrared region (NIR) absorption (650-900 nm) have numerous
58 advantages in imaging studies on living organisms because they can penetrate more
59 deeply into tissues and auto-fluorescence is not observed in this region [11]. Such
60 properties make heptamethine cyanine dyes one of the most important NIR dyes, being
61 widely applied as fluorescent labels in imaging studies [12]. Fluorescence sensors based
62 on cyanine dyes have been used for NIR-sensing of small organic molecules and metal
63 ions, however, studies on the use of NIR-cyanine based probes in the detection of Cu^{2+}
64 metal ions are scarce [1], [13], [14].

65 Despite the versatile photophysical properties of cyanines, their incorporation into
66 polymers is also very rare [15]. Combining the superior properties of cyanines with
67 polymers can result in an ideal platform for the development of pH-sensors with long-
68 term stability.

69 Stimuli-responsive polymer-based optical sensors, especially the ones used for pH and
70 temperature sensing, have attracted great attention because of their fast response time
71 and reversibility [16].

72 Precise control over the acidity or basicity is essential in many areas such as (i) chemical,
73 cosmetic and household industries; (ii) for environmental control (i.e. remediation) and
74 (iii) in life sciences [17].

75 With the growth of the chemical industry, strong acids such as hydrochloric acid, sulfuric
76 acid, hydrofluoric acid and perchloric acid are commonly used in almost all industries
77 worldwide [18].

78 Nowadays, pH measurements are performed in aqueous environments on a pH scale
79 from 1 to 13, by using pH test strips, potentiometric techniques and chemical pH sensors.
80 However, there is a lack of devices that allow the rapid, precise and inexpensive

81 measurement of pH outside this scale, specifically at very low pH values [19]. The
82 development of pH-responsive polymeric materials containing optical sensors has been
83 shown as an approach to overcome this issue.

84 Herein, three cyanine derivatives **1**, **2** and **3** were explored as optical sensors for the
85 detection of Cu^{2+} , Cd^{2+} , Hg^{2+} , Ni^{2+} , Co^{2+} , Zn^{2+} , Ag^+ metal ions in aqueous media. Based
86 on the results obtained in solution, polymethylmethacrylate (PMMA) polymers containing
87 cyanine or Cu^{2+} @cyanine complex were synthesized and studied as low pH and
88 temperature sensors.

89 **2. Experimental Section**

90 **2.1. Chemicals and starting materials**

92 2-chloro-3 (phenylamino) allylidene) benzenaminium chloride) was used as a
93 commercial product purchased from ORGANICA Feinchemie GmbH Wolfen. Copper(II)
94 trifluoromethanesulfonate ($\text{Cu}(\text{TfO})_2$, > 98%), cadmium(II) trifluoromethanesulfonate
95 ($\text{Cd}(\text{TfO})_2$, > 98%), mercury(II) trifluoromethanesulfonate ($\text{Hg}(\text{TfO})_2$, > 98%), nickel(II)
96 trifluoromethanesulfonate ($\text{Ni}(\text{TfO})_2$, > 98%), cobalt(II) trifluoromethanesulfonate
97 ($\text{Co}(\text{TfO})_2$, > 98%), zinc(II) trifluoromethanesulfonate ($\text{Zn}(\text{TfO})_2$, > 98%) and silver(I)
98 trifluoromethanesulfonate ($\text{Ag}(\text{TfO})$, > 98%) were acquired from Solchemar.

99 Polymethyl methacrylate (PMMA) and hydrochloric acid (HCl, 37%) were purchased
100 from Sigma Aldrich. Dichloromethane (DCM) was purchased from Honeywell.
101 Acetonitrile (ACN) was produced by Carlo Erba Reagents. Perchloric acid (HClO_4 , 20%)
102 was bought from Panreac Applichem. Hydrobromic acid (HBr, 45%) and ortho-
103 phosphoric acid (H_3PO_4 , 85%) were acquired from Alfa Aesar. Formic acid (HCOOH,
104 LC-MS grade) and methanesulfonic acid ($\text{CH}_3\text{SO}_3\text{H}$ > 98%) were purchased from
105 Thermo Scientific. All solvents and reagents were of analytical reagent grade and were
106 used as received.

107 **2.2. Synthetic procedures**

109 The chemical structure of dye **1** was confirmed by a combination of spectroscopic
110 techniques including ¹H-NMR, ¹³C-NMR and HRMS (Fig. S1-S9). The ¹H- and ¹³C-NMR
111 spectra were recorded on a Bruker Avance II+ 600 at 600 MHz and 151 MHz,
112 respectively, in DMSO-d₆ at 25 °C. The signal at 2.50 ppm (¹H-NMR) and at 39.52 ppm
113 (¹³C-NMR) were used as an internal reference for the DMSO-d₆. Chemical shifts (δ) are
114 expressed in ppm, whereas *J* coupling constants are expressed in Hz (0.1 Hz accuracy).
115 The following acronyms were used to describe the spin multiplicity in ¹H-NMR: s = singlet,
116 d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet
117 of triplets. Mestrelab Research S.L.'s MestreNova v. 14.1.1 was used to process the raw
118 NMR data files. HRMS of the dye **1** was acquired on Q Exactive Plus Orbitrap Mass
119 Spectrometer, Thermo Scientific. Dye **2** was prepared according to a method reported in
120 the literature [9]. Dye **3** was prepared according to reference [20].

121

122 2.2.1. Synthesis of the cyanine dyes:

123 3-butyl-2-methylbenzo[d]thiazol-3-ium iodide [21], [22] (1 equiv., 333 mg, 1 mmol) and
124 the commercial product 2-chloro-3-(phenylamino)allylidene)benzenaminium chloride
125 (1.5 equiv., 440 mg, 1.5 mmol) from ORGANICA Feinchemie GmbH Wolfen, were mixed
126 with 5 mL methanol in a mortar with a pestle. After homogenization of the mixture, sodium
127 acetate (2 equiv., 164 mg, 1 mmol) was added. The reaction mixture was grinded for 5
128 minutes at room temperature, which resulted in the crystallization of a solid product. The
129 crude product was dissolved in 10 mL methanol and transferred to a beaker containing
130 200 mL of an aqueous solution of potassium iodide (3 equiv., 498 mg, 3 mmol). A
131 precipitate formed, and was collected by filtration under reduced pressure. Analytical
132 samples were obtained after repeated recrystallization from methanol, yielding 332 mg
133 (66.9%) of dark red solid with metallic shine (m.p. = 213-215 °C). The chemical structure
134 of the target dye **1** was confirmed by ¹H-NMR, ¹³C-NMR and HRMS (ESI in both positive
135 and negative modes).

136 ¹H NMR (600 MHz, DMSO-d₆) δ 10.52 (s, 1H, NH), 8.75 (s, 1H), 8.30 – 8.24 (m, 2H),
137 8.06 (d, *J* = 8.4 Hz, 1H), 7.77 – 7.69 (m, 1H), 7.61 (t, *J* = 7.7 Hz, 1H), 7.52 – 7.47 (m,
138 2H), 7.44 (dd, *J* = 8.6, 7.2 Hz, 2H), 7.19 (tt, *J* = 7.3, 1.2 Hz, 1H), 6.67 (d, *J* = 13.8 Hz,
139 1H), 4.63 (t, *J* = 7.5 Hz, 2H), 1.89 – 1.64 (m, 2H), 1.55 – 1.31 (m, 2H), 0.94 (t, *J* = 7.4
140 Hz, 3H). ¹³C NMR (151 MHz, DMSO-d₆) δ 169.2, 148.2, 145.6, 141.2, 139.6, 129.6,
141 128.7, 126.8, 126.4, 124.8, 123.8, 117.7, 115.3, 107.6, 100.7, 47.2, 29.9, 19.3, 13.6.
142 HRMS-ESI(+): *m/z*: Found 369.1187 [M⁺] C₂₁H₂₂ClN₂S⁺ ; Requires [M⁺] 369.1187 /
143 HRMS-ESI(-) [I⁻] Requires 126.9050 , *m/z*: Found 126.9037 [I⁻].

144 The symmetrical NIR heptamethine cyanine dyes **2** and **3** were prepared according to
145 the methods reported in the literature [9], [20] .

146

147 **2.3. Spectrophotometric and spectrofluorimetric measurements**

148 UV-Vis absorption spectra were recorded with a JASCO V-650 spectrophotometer and
149 a fluorescence emission by a HORIBA Scientific FLUOROMAX-4 spectrofluorimeter.
150 The linearity of the fluorescence emission vs. the concentration was checked in the
151 concentration used (10⁻⁴ – 10⁻⁶ M). A correction for the absorbed light was performed
152 when necessary. Spectra of solid samples were collected with a Horiba-Jobin-Yvon
153 Fluoromax-4[®] spectrofluorometer using an optic fibre connected to the equipment, by
154 exciting the solid compounds at appropriated λ (nm).

155 **2.3.1. Photophysical characterization and titrations**

156 The spectroscopic characterizations and titrations were performed using stock solutions
157 of the compounds (ca. 10⁻³ M), prepared by dissolving the appropriate amount of dyes **1**
158 to **3** in acetonitrile, and acetonitrile: MilliQ water (1:1) in a 4 mL volumetric flask. The
159 studied solutions were prepared by appropriate dilution of the stock solutions up to 10⁻⁵
160 – 10⁻⁶ M. Titrations of dyes **1** to **3** were carried out by the addition of microliter amounts
161 of standard solutions of Cu²⁺, Cd²⁺, Hg²⁺, Ni²⁺, Co²⁺, Zn²⁺, Ag⁺ ions in acetonitrile. All
162 measurements were performed at 298 K. The stability constants were determined using

163 the HypSpec program [23].

164 Luminescence quantum yield of dye **1** was measured using a solution of Rhodamine B
165 in ethanol [$\phi_F = 0.70$] as a standard [24], [25]. All solvents used were of the highest purity
166 from Merck.

167

168 **2.4. Determination of the detection and quantification limits (LOD and LOQ)**

169 The detection limit (LOD) and quantification limit (LOQ) are the lowest concentration
170 levels that can be detected and quantified to be statistically different from a blank. For
171 the determination of LOD and LOQ, ten different measurements of a solution containing
172 the selected probe were collected without the addition of any metal ion (y_{blank}). The LOD
173 and LOQ were determined by the formulas:

174 $\text{LOD} = y_{\text{dl}} = y_{\text{blank}} + 3\text{std}$, where y_{dl} = signal detection limit and std = standard deviation.

175 $\text{LOQ} = y_{\text{dl}} = y_{\text{blank}} + 10\text{std}$, where y_{dl} = signal detection limit and std = standard deviation.

176 Additionally, small amounts of the metal ion were added to a solution of the cyanine dyes
177 **1** to **3**, to determine the minimal detectable and quantified concentration out of the LOD
178 and LOQ values, respectively.

179 **2.5. Dye doped Polymeric films**

180 The general workflow for the preparation and study of the cyanine-doped
181 polymethylacrylate (PMMA) polymers is shown in Figure 1. The cyanine-doped PMMA
182 polymers were obtained, at room temperature, by dissolving 100 mg of PMMA in 2 mL
183 dichloromethane followed by the addition of 2 mg of cyanine dyes **1** to **3**, previously
184 dissolved in another 2 mL of dichloromethane. The polymer films were obtained after
185 very slow evaporation at room temperature (~48 hours). Non-doped PMMA films were
186 prepared as controls using the same procedure.

187 Each film was cut in approximately 1 x 1 cm pieces to perform the assays with
188 temperature and acids. PMMA polymers of each cyanine dye were also made with the
189 addition of 3 equivalents of Cu(TfO)₂, following the same protocol described above.

190 2.5.1. Temperature sensing

191 The polymer pieces were placed between two quartz slides and the emission spectra
192 were collected using an optical fiber directly connected with the spectrophotometer. The
193 measures were performed from 25 to 170°C and from 170 to 25°C. Emission spectra
194 were acquired at intervals of 5°C and 10°C for each polymer.

195 2.5.2. Acidity Assays

196 The reactivity and resistance of the dye-doped polymers to strong acids was tested by
197 dipping ca. 0.5 x 0.5 cm pieces in a 2 – 12 M concentration gradient of HCl, HBr, HClO₄,
198 H₃PO₄, HCOOH and CH₃SO₃H solutions, for 10 mins. Time-dependent behavior, in HCl,
199 was also assessed for **1** to **3** dye-doped polymers, by dipping them for 15, 30, 45, 60,
200 120, and 180 min in 12 M HCl solutions. Any changes in the polymers were observed
201 either optically or spectroscopically, using the same setup described in the temperature
202 sensing assay.

203 *Insert Figure 1.*

204

205 The potential of PMMA_3 and PMMA_3@Cu²⁺ to act as low pH sensor was further
206 tested. Samples of both polymers were first activated in 12 M HCl for 3 hours:
207 subsequently, their ability to change reversibly from green (when dry) to yellow (when
208 submerged in acid) was tested with consecutive cycles of drying and dipping the
209 polymers in 12 M HCl (see video 1, supplementary materials). In an additional test, after
210 activation for 3 hr in 12 M HCl, samples of PMMA_3 and PMMA_3@Cu²⁺ were dried
211 (turning green) and consecutively dipped in HCl solutions of decreasing concentration
212 (12 M, 11 M, 10 M, 8 M, 6 M, 4 M and 2 M); colour change was observed up to HCl 6 M.

213 **3. Results and Discussion**

214 **3.1. Synthesis**

215 The novel unsymmetrical cyanine dye **1** was successfully prepared via a new synthetic
216 protocol, using mild reaction conditions; the scheme of reaction is depicted in Figure 2.
217 For the synthesis, 1 equiv. of the *N*-quaternary benzothiazolium salt (3-butyl-2-
218 methylbenzo[d]thiazol-3-ium iodide) [21], [22] and an excess of the conventional dianilide
219 linker (2-chloro-3-(phenylamino)allylidene)benzenaminium chloride) were used. The two
220 starting components were grinded together in a mortar using minimum amount of polar
221 protic solvent – methanol as the reaction media. Then, 2 equiv. of sodium acetate were
222 used as a mild basic reagent. All steps were carried out at room temperature. The target
223 compound was isolated in good yield (66.9 %) yield as dark red crystals with metallic
224 shine. The identity of the chemical structure was confirmed by various spectroscopic
225 techniques such as NMR and high-resolution mass spectrometry.

226 *Insert Figure 2.*

227 As described in the Experimental section, the symmetrical NIR heptamethine cyanine
228 dyes **2** and **3** were prepared according to the methods reported in the literature [9], [20].

229 **3.2. Photophysical characterization**

230 Absorption and emission spectra of cyanine derivatives **1** to **3** were performed in
231 acetonitrile solutions, at 298 K; these are presented in Figure 3.

232 *Insert Figure 3*

233 Figure 3 (A) shows the absorption spectra of cyanine derivatives **1** to **3**, with maximum
234 bands located at 484 nm, 803 nm and 774 nm, respectively, characteristic of π - π^*
235 transitions. Heptamethine cyanines **2** and **3** present longer excitation wavelengths, with
236 emission bands located at the NIR region (823 nm for **2** and 800 nm **3**), proving that

237 structural modifications at the polymethine bridge can easily tune the absorption and
238 emission bands (Fig. 3 B). Both compounds **2** and **3** have a green naked-eye colour;
239 they are not emissive under a UV-lamp, and they emit in the NIR region. Compound **1**
240 has an emission band at 541 nm, presenting a yellow-brown naked-eye colour and a
241 yellow colouration under a UV-lamp. Cyanine **1** has the lowest molar extinction
242 coefficient ($\epsilon = 4.17 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$) possible due to bearing a hemicyanine backbone
243 structure (Table 1). The presence of a chlorocyclohexene group in the polymethine chain
244 of heptamethine cyanines **2** and **3** gives stability to the benzothiazole and trimethylindole
245 moieties in the ground state [12], which results in higher molar extinction coefficients (ca.
246 $\approx 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$) (Table 1). According to the literature [26], in acetonitrile, dye **3** has a
247 fluorescence quantum yield (ϕ) value of 0.11 ($\lambda_{\text{exc.}} = 774 \text{ nm}$ and $\lambda_{\text{emis.}} = 800 \text{ nm}$); dye **2**
248 [9] has a value of $\phi = 0.12$ ($\lambda_{\text{exc.}} = 803 \text{ nm}$ and $\lambda_{\text{emis.}} = 823 \text{ nm}$). The fluorescence
249 quantum yield (ϕ) of dye **1** was determined using as a standard a solution of rhodamine
250 B in ethanol ($\phi = 0.70$), having a calculated value of $\phi = 0.01$.

251

Insert Table 1

252 **3.3 Sensing ability to Metal Ions in solution**

253 The sensorial ability of cyanine dyes **1** to **3** towards Cu^{2+} , Cd^{2+} , Hg^{2+} , Ni^{2+} , Co^{2+} , Zn^{2+} ,
254 Ag^+ metal ions in acetonitrile, was evaluated by titrating the free ligand with small
255 amounts of the metal ions. The absorption and emission spectra were collected at 298
256 K until reaching a plateau. From all studied metal ions, all cyanine dyes showed high
257 selectivity and sensitivity to Cu^{2+} metal ions, even in the presence of other metal ions.
258 Competitive studies revealed that the other metal ions have no interference (see figure
259 S10, supplementary material).

260 Figure 4 shows the absorption and emission spectral changes of **1**, **2** and **3** upon
261 coordination with Cu^{2+} metal ions, in acetonitrile.

262

Insert Figure 4

263 As shown in Fig. 4A, the solution of free **1** exhibits an absorption band at 484 nm,
264 responsible for its yellow colour. When increasing Cu²⁺ concentration, the absorption at
265 484 nm increases and shifts to a longer wavelength (500 nm), followed by a decrease in
266 absorbance and a consequent loss of colour, going from yellow to colourless. In the
267 emission spectra (Fig. 4B), an increase in the emission intensity at 541 nm is detected,
268 until the addition of 1 equivalent of Cu²⁺, further decreasing with higher Cu²⁺
269 concentrations. The quenching of the emission signal is due to the paramagnetic of Cu²⁺
270 metal ions. The addition of Cu²⁺ metal ions to heptamethine cyanine dyes **2** and **3**
271 promoted significant changes in the ground and excited states. In both cases, a decrease
272 in the absorbance was observed at 803 nm (**2**) and 774 nm (**3**). These spectral
273 modifications are in agreement with the observed colour changes, going from its
274 characteristic light green to pink (**2**) and violet (**3**). Concerning the emission intensity of
275 both dyes, a quenching of the emission signal at 823 nm (**2**) and 800 nm (**3**) is observed.

276 In all cases, the coordination of the Cu²⁺ metal ions occurs in the nitrogen and sulfur
277 atoms of the benzothiazole and trimethylindole heterocycles, with the coordination at the
278 nitrogen atom usually leading to an increase in the emission. In fact, in compound **1** the
279 addition of 1 equivalent of Cu²⁺ increases the emission signal, probably due to the
280 coordination of Cu²⁺ to the secondary amine in the polymethine chain, avoiding then the
281 photoinduced transfer process. However, the paramagnetic nature of the Cu²⁺ leads to
282 non-radiative deactivation processes [27], [28], and thus a quenching in the emission
283 intensity is observed in all cases.

284 To improve the potential application of these dyes to biological environments, the titration
285 of all the dyes with Cu²⁺ metal ions was also carried out in an aqueous solution of
286 acetonitrile:milliQ water (1:1). The spectrophotometric titrations are depicted in Figure 5.

287 The increase in the proportion of water produces slight changes in the absorption and
288 emission maximum bands in all compounds, with the most noticeable one in cyanine **1**,

289 where the absorption maximum band is red-shifted 20 nm. However, similar to what was
290 observed in acetonitrile, in aqueous mixtures, the addition of copper (II) ions induces the
291 same spectral and colour changes.

292 *Insert Figure 5*

293 Despite keeping the same spectral changes in the ground state, the increased presence
294 of water in the solution led to an evident quenching in the emission intensity of dyes **2**
295 and **3**. Nonetheless, dye **1** remains emissive with a maximum emission band centred at
296 543 nm. Moreover, in the absorption spectra of dye **3**, an initial plateau is observed with
297 the addition of Cu²⁺ in acetonitrile, but not in the CH₃CN:H₂O (1:1) solution. The plateau
298 could be attributed to the preference of Cu²⁺ coordination to acetonitrile molecules rather
299 than the nitrogen atom in the indole moiety, being such preference eliminated with the
300 addition of water molecules.

301 The stability constants, as well as the minimal detectable and quantified amount of Cu²⁺
302 in acetonitrile and in aqueous mixtures (*), were calculated for all compounds and the
303 data is summarized in table 2.

304 *Insert Table 2*

305 The stability constants reveal the formation of mononuclear species in both solvents,
306 where the strongest interaction was obtained in acetonitrile for NIR cyanine **2** (Table 2).
307 In general, NIR cyanines revealed stronger interactions with Cu²⁺ in acetonitrile than in
308 acetonitrile:water medium.

309 These results are in agreement with the minimal detectable and quantified amounts of
310 Cu²⁺ by these cyanine derivatives, with the lowest amount also being obtained for dye **2**.
311 Thus, cyanine **2** is able to detect and quantify the lowest amount of 0.9/1.6 μM, in
312 acetonitrile, and of 0.3/0.6 μM in aqueous medium.

313

314 **3.3. Dye-doped PMMA films for pH and temperature sensing**

315 Aiming to explore deeper the wider applications of these cyanine dyes, several low-cost
316 cyanine-doped and copper@cyanine-doped PMMA polymers were prepared and
317 studied as low pH and temperature sensors. Figure 6 depicts the obtained PMMA-doped
318 samples.

319 *Insert Figure 6*

320 All PMMA doped samples showed a reasonable degree of homogeneity, suggesting
321 good compatibility between the polymeric matrix and the dye, or complex. The films were
322 characterized by solid-state emission. PMMA_1 showed an emission band at 550 nm,
323 with a light orange colour at both white light and UV-light (365 nm). PMMA_1@Cu²⁺ had
324 a more intense orange colour, with a maximum emission band at 591 nm. PMMA_2 and
325 PMMA_2@Cu²⁺ have blue and green naked-eye colour respectively, with PMMA_2
326 having no detectable emission under UV light (the solid-state emission spectra showed
327 a very weak band at 824 nm) and PMMA_2@Cu²⁺ having a green emission (λ_{em} = 510
328 nm).

329 The films PMMA_3 and PMMA_3@Cu showed similar naked-eye colours, the latter with
330 a more intense green colour. Both polymers did not show any significant emissions.

331 **3.3.1. Temperature sensing**

332 The PMMA-doped samples were also used to ascertain their potential thermal sensing
333 capability. To determine their potential applications as thermal sensors the films were
334 placed on a heating plate and subjected to temperature variations, heating from 25 to
335 170°C and then cooling from 170 to 25°C. Emission spectra were acquired at intervals
336 of 5°C and 10°C for each polymer. Figure S11 depicts the emission spectra variation of
337 PMMA_1 and PMMA_1@Cu²⁺ with temperature.

338 Whereas heating from 25°C to 170°C led to a quenching of the emission intensity in both
339 polymers (PMMA_1 and PMMA_1@Cu²⁺), cooling from 170°C to 25°C allowed a total
340 recovery of the emission intensity for PMMA_1@Cu²⁺ and of ca. 70% for PMMA_1.

341 After being exposed to the same temperature variations, PMMA_2 showed a naked-eye
342 colour change from green to red, accompanied by the surgency of emission in the red
343 region (see Fig. 7). For PMMA_2@Cu²⁺, a change of colour from green to brown was
344 also observed, followed by an increase in its green emission (see Fig. 7). These
345 modifications revealed to be irreversible, and no return to its original color after the
346 cooling cycle was observed. Based on these colour changes, emission spectra with
347 temperature were followed at 824 nm and 660 nm, due to the detected red emission in
348 PMMA_2, after heating (see Fig. 7). In the case of PMMA_2@Cu²⁺ emission spectra
349 were followed at 510 nm and 824 nm.

350

Insert Figure 7

351 PMMA_2 showed similar spectral behaviour at both 824 nm and 660 nm, where a
352 quenching in the emission intensity (ca. 20%) is observed at 140°C, recovered after
353 cooling. The recovery at 660 nm is ca. 125%. This fluorescence “switch on” is possibly
354 due to the increase of interactions between the dye and the acrylate moieties of the
355 polymer, increasing dye planarity. An increment in the emission intensity (ca. 30%) at
356 510 nm, is observed in the PMMA_2@Cu²⁺ film during the heating phase. This rise in
357 intensity was irreversible, even after cooling, with no detectable optical changes.

358 In the same way, PMMA_3 and PMMA_3@Cu²⁺ have colourimetric and fluorimetric
359 irreversible changes upon heating, with colour changing from green to brown (PMMA_3),
360 or greenish-brown (PMMA_3@Cu²⁺), and the appearance of a green emission (see Fig.
361 8). Emission spectra were followed at 824 nm and 550 nm (see Fig. 8). Similarly to what
362 was observed with dye 2, quenching in the emission intensity is detected above 120 °C,
363 followed by a recovery of 160% of its emission.

364 Overall, PMMA_1 and PMMA_1@Cu²⁺ are reversible fluorimetric thermoresponsive
365 polymers, while PMMA_2, PMMA_3 PMMA_2@Cu²⁺ and PMMA_3@Cu²⁺ are
366 irreversible colourimetric and fluorimetric thermoresponsive polymers.

367 *Insert Figure 8*

368 3.3.2. pH sensing

369 Having in mind the lack of devices for rapid, precise and inexpensive measurements of
370 pH out of the 1-13 scale, especially at very low pH [19], the PMMA-doped samples were
371 exposed to 12 M HCl solutions for increased periods of time, from 15 min to 180 min (3
372 hours). Most polymers showed a degree of spectral changes after 3 hours, be it emission
373 maximum shifts, emission enhancement or colour change.

374 For PMMA_1 and PMMA_1@Cu²⁺, although no visible changes in colour or emission
375 under a 365 nm UV-lamp are perceived, keeping their characteristic orange colour and
376 emission, spectral analysis reveals an increase in the emission of PMMA_1 ($\lambda_{\text{max}} = 543$
377 nm) and a combined increase and blue shift of the emission maxima of PMMA_1@Cu²⁺,
378 from 592 nm to 543 nm (Figure 9). This shift suggests that the presence of protons
379 interferes with the coordination of Cu²⁺ ions in the dye-copper complexes of the polymer,
380 making PMMA_1 and PMMA_1@Cu²⁺ polymers approximately similar, after their
381 immersion in acid.

382 *Insert Figure 9*

383 Of all six PMMA-doped samples, only PMMA_2@Cu²⁺, PMMA_3 and PMMA_3@Cu²⁺
384 showed significant colourimetric and/or fluorimetric changes, after 2 hours of exposure
385 to the selected HCl concentration. For PMMA_2@Cu²⁺ only a change in colour from
386 green to brown is visible with no reversion to its original colour after total dryness. For
387 both PMMA_3 and PMMA_3@Cu²⁺ a visible change from green to yellow is

388 accompanied by the surgency of an emission band at 805 nm (Figures 10 and Figure
389 S11).

390 After activation of PMMA_3 and PMMA_3@Cu²⁺ with 12M HCl for 3 hours, their ability
391 to change from green (when dry) to yellow (when in acid) was further tested with
392 consecutive cycles of drying and dipping the polymer in 12 M HCl. For both, recorded
393 changes displayed a consistent and almost immediate reversible behaviour when
394 removed from the acid solution, even after 10 cycles (see video 1, supplementary
395 material).

396 *Insert Figure 10*

397 One of the pH sensing mechanisms reported for pH probes based on a cyanine scaffold
398 arises from non-N-alkylated indolium moieties, in which protonation and deprotonation
399 could occur depending on proton concentration. As reported in the literature [12], if an
400 indole nitrogen atom of a cyanine dye is not alkylated, such dye is highly sensitive to pH
401 variations. Acidic conditions lead to the protonation of the indole nitrogen atoms inducing
402 a strong fluorescence, which is reversed at basic conditions where the nitrogen is
403 deprotonated, and the emission quenched. Such behaviour is advantageous when
404 designing colourimetric pH-dependent probes, allowing for naked-eye detection of pH
405 variations.

406 Thus, for PMMA_3 and PMMA_3@Cu²⁺, differently from dyes 1 and 2, the low N-
407 alkylation (methyl group) of the indolium moiety allows for a degree of protonation of the
408 indole nitrogen atom and providing the polymer with pH sensing properties, with the first
409 2 hours working as activation time. In brief, the presence of the indoline ring can
410 compromise the delocalization of the π -electrons rendering the lone-pair of the nitrogen
411 atom with a higher capability to be protonated. This change renders the dye with a H⁺
412 ion sensing capacities. After this period, the doped polymers can detect pH variations in
413 seconds (video 1, supplementary material). Following a 3 hours activation in 12 M HCl,

414 both PMMA_3 and PMMA_3@Cu²⁺ were also capable of effectively sensing 8 M, 10 M,
415 11 M HCl solutions, as well as 12 M, changing their color from green to yellow and having
416 an increase in their emission maxima at 805 nm (see Figure S13, supplementary
417 material).

418 Interestingly, at concentrations of 6 M and 4 M the polymers remain green, or even turn
419 to darker green, as is the case of PMMA_3. In line with the above-mentioned mechanism,
420 this suggests that at these concentrations the pressure of proton excess in media is not
421 enough to force a protonation of the indole nitrogen atom, hampering their pH sensing
422 capacity at these lower concentrations. To support this, activation of PMMA_3 and
423 PMMA_3@Cu²⁺ in milliQ water, followed by immersion in the same set of HCl
424 concentrations (4 to 12 M) produced no changes in both polymers. This rules out
425 hydration as the underlying activating mechanism, confirming that low proton
426 concentrations are not enough to induce protonation of the indole nitrogen, thus
427 rendering both polymers with specificity to superacid conditions.

428 Thus, with a capacity to sense high concentrations of acid and an outstanding reversible
429 behaviour, PMMA_3 and PMMA_3@Cu²⁺ are serious candidates for superacid sensing,
430 which could allow for a real-time follow up of contaminated effluents, or their
431 incorporation in quality control proceedings in industrial processes

432 Taking into consideration the properties of PMMA_3 and PMMA_3@Cu²⁺, both polymers
433 were tested against HBr, HClO₄, H₃PO₄, HCOOH and CH₃SO₃H solutions, in a set of
434 concentrations of 2 M, 4 M, 6 M, 8 M, 10 M and 12 M. Contrary to the results registered
435 above, no changes were seen in PMMA_3 through most acids, with some even
436 degrading the polymer in the process, making its applicability null. Regarding
437 PMMA_3@Cu²⁺, similar responses were obtained to the same acids, apart from 12 M
438 formic acid, where the polymer shifted from its initial green colour to a blue tonality,
439 working as well as a colourimetric, but irreversible probe.

440 **4. Conclusions**

441 Cyanine derivatives **1** to **3** were successfully characterized and their sensing abilities
442 towards metal ions explored, revealing an unprecedented colourimetric selectivity
443 towards Cu²⁺ metal ions, with detection and quantification of the lowest amounts of 0.3
444 and 0.6 μM in aqueous solution. Incorporation of their cyanine's derivatives and their
445 copper complexes into PMMA polymers led to polymer-based optical sensors with
446 superior properties. As temperature sensors, PMMA_1 and PMMA_1@Cu²⁺ are
447 reversible fluorimetric thermoresponsive polymers; PMMA_2, PMMA_3 PMMA_2@Cu²⁺
448 and PMMA_3@Cu²⁺ are irreversible colourimetric and fluorimetric thermoresponsive
449 polymers. As low pH sensors, PMMA_3 and PMMA_3@Cu²⁺ polymers are promising
450 candidates for superacid sensing, after activation, both polymers allow for the naked-eye
451 detection of strong acids *via* colourimetric changes in seconds. The results obtained
452 could greatly contribute for the development of intelligent tags for use in industry and
453 work safety, directed to Cu²⁺ metal ions, acidity and temperature control in health, in the
454 environment, industrial processes and laboratories.

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