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1 **Generation and Characterization of Air Micro-bubbles in**  
2 **Highly Hydrophobic Capillaries**

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11  
12 **Keywords:** Apparent charge / Electrophoretic mobility / Micro-bubbles generation /  
13 Superhydrophobic coatings

14 **Abbreviations:** BGE Background Electrolyte / CE Capillary Electrophoresis / DMF  
15 *N,N*-Dimethylformamide / GC Gas Chromatography / HEPES acide 4-(2-  
16 hydroxyéthyl)-1-pipérazine éthane sulfonique / I.D. Internal Diameter / NaOH Sodium  
17 hydroxyde

18

## 19 **Abstract**

20 The generation of air microbubbles in microfluidic systems or in capillaries could be  
21 of great interest for transportation (single cell analysis, organite transportation) or for  
22 liquid compartmentation. The physicochemical characterization of air bubbles and a  
23 better understanding of the process leading to bubble generation during  
24 electrophoresis is also interesting in a theoretical point of view. In this work, the  
25 generation of microbubbles on hydrophobic Glaco<sup>TM</sup> coated capillaries has been  
26 studied in water-based electrolyte. Air bubbles were generated at the detection  
27 window and the required experimental parameters for microbubbles generation have  
28 been identified. Generated bubbles migrated against the electroosmotic flow, as  
29 would do strongly negatively charged solutes, under constant electric field. They  
30 have been characterized in terms of dimensions, electrophoretic mobility, and  
31 apparent charge.

## 32 **1 Introduction**

33 Gas bubbles are used for numerous applications: contrast agents for medical  
34 imagery by ultrasounds [1–3], therapeutic drug delivery by encapsulating the drug  
35 into the bubble [4–6], wastewater treatment [7], surface cleaning [7–9], froth flotation  
36 [10–12], drag reduction [9], sterilization of bacteria [13], enhanced germination rate  
37 of plant seeds [14], promotion of physiological activity of living organisms [15], and  
38 improved blood oxygenation [16]. Usually, air microbubbles are produced by  
39 mechanical agitation [17,18], or by increasing the temperature of cold (4°C) gas  
40 saturated water [19–21]. The presence of the nano or micro air bubbles in the liquid  
41 bulk can be observed using a laser beam via light scattering (Tyndall effect). The  
42 control of the air bubble size can be challenging but is still very important for the  
43 applications. The production of gas bubbles onto surfaces can be obtained by  
44 changing the solvent, for instance, by covering a surface with ethanol, and then  
45 slowly replacing the ethanol by water. The two liquids must be saturated with air or  
46 any other gas. Since ethanol has a higher gas solubility compared to water, a  
47 transient gas oversaturation occurs and the excess of gas results in the apparition of  
48 air bubbles onto the surface [22]. Another possibility to produce bubbles is to replace  
49 water with salted water, but the mechanism behind this phenomenon is not clear,

50 since air bubbles form when water is replaced by salted solution, but also when  
51 salted solution is replaced by water [23]. Another simple way to produce bubble onto  
52 a surface is by simply immersing the surface into water. When a dry surface is put in  
53 contact with a water droplet, there is an air pressure enhancement between the  
54 surface and the water droplet, leading to the formation of a dimple in the droplet,  
55 resulting in an air bubble [24]. This phenomenon is favored by the surface  
56 roughness/irregularities, as observed on superhydrophobic surfaces used in our  
57 study.

58 In capillary electrophoresis or in microfluidics in general, the presence of bubbles is  
59 usually not desired because it tends to destabilize the electrical current and to limit  
60 the repeatability of the separations [25]. It is therefore important to know in which  
61 conditions air bubbles can be formed and how to avoid them experimentally [25]. On  
62 the other hand, the generation of air microbubbles in microfluidic systems or in  
63 capillaries could be of great interest for transportation (single cell analysis, organites)  
64 [26,27] or for liquid compartmentation [28]. In this case, it is important, for practical  
65 reasons, to control the generation of the air bubbles [26]. The characterization of air  
66 bubbles, in terms of size, surface (or interface) charges, velocities or electrophoretic  
67 mobilities, is also crucial since these characteristics are leading their behavior and  
68 their fate in the environment in which they are formed/placed. The electrokinetic  
69 characteristics of air bubbles have been studied both theoretically [29,30], and  
70 experimentally [19,31,32]. Generally, negative zeta potentials were measured for air  
71 bubbles in water based electrolytes [19,31,32], except when cationic surfactant are  
72 present in the electrolyte leading to a reverse of the sign of the charge [19,32]. The  
73 origin of the negative charge is still under debates, but different explanations were  
74 proposed: an excess of hydroxide anions near the vapor/water surface [33,34];  
75 Vacha et al. [35] and later Leroy et al. [30] suggested that the surface of the water  
76 molecules become partially charged due to a lack of balance between the number of  
77 donating and accepting hydrogen bonds. Zeta potential were derived from  
78 electrophoretic mobility measurements, generally performed by laser Doppler  
79 electrophoresis [19,32].

80 In a previous study [36], it was shown that Glaco<sup>TM</sup> coated capillaries led to  
81 superhydrophobic capillaries (with meniscus inversion compared to fused silica  
82 capillary and slipping conditions at the capillary surface). The existence of a

83 nanometric air layer at the interface between the coating and the aqueous phase in  
84 the Glaco™ capillaries was proved by ellipsometry [36] and was also put in evidence  
85 by the ‘mirror’ effect observed on a Glaco™ coated blade immersed in water. These  
86 superhydrophobic capillaries were obtained by the deposition of a 1 μm thickness  
87 film of Glaco™ solution (containing nanostructured hydrophobically modified silica  
88 nanoparticles) by flushing the 50 μm I.D. × 40 cm capillary previously filled with the  
89 Glaco™ solution with air at a constant pressure of 100 psi [36]. The film of Glaco™  
90 solution was next dried in a GC-oven. However, the resulting superhydrophobic  
91 capillaries were hardly filled with water-based electrolyte, leading to a ‘train’ of air  
92 bubbles in the capillary and to unstable current intensity in CE [36]. Stable current  
93 intensities were only obtained after a few electrophoretic runs, when the  
94 superhydrophobicity was reduced.

95 In this work, the use of 80 psi instead of 100 psi to flush the capillary previously filled  
96 with the Glaco™ solution during the coating protocol leads to slightly thinner Glaco™  
97 liquid film deposition (0.9 μm) onto the capillary surface, and thus to slightly lower  
98 superhydrophobicity. The 80 psi-Glaco™ coated capillaries can be easily filled with  
99 water-based electrolyte and lead to stable current intensity in CE. The generation of  
100 air bubbles inside such capillary has been studied in water based electrolyte. Air  
101 bubbles were generated at the detection window and the required parameters for  
102 micro-bubbles generation have been identified. Generated bubbles were displaced  
103 by electrophoretic migration under constant electric field and have been  
104 characterized in terms of dimensions, electrophoretic mobility, and apparent charge.

105

## 106 **2 Materials and methods**

107

### 108 **2.1 Chemicals**

109 Glaco Mirror Coat Zero™ was purchased from Soft99 (Osaka, Japan). It is  
110 composed of silica nanoparticles suspension (hydrophobically modified with alkyl  
111 groups) dispersed in isopropanol. The nanoparticle concentration in the Glaco™  
112 solution is about 4 g.L<sup>-1</sup>, as quantified by weighing the dry residue after evaporation  
113 of the solvent. Ultrapure grade water (18 MΩ.cm<sup>-1</sup>) was produced on a Milli-Q

114 equipment (Merck Millipore, USA). Sodium hydroxide (NaOH) and *N,N*-  
115 dimethylformamide (DMF, 99%) were purchased from Sigma-Aldrich (St Quentin  
116 Fallavier, France). 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) was  
117 purchased from VWR (Fontenay-sous-bois, France). Brilliant Blue FCF (food  
118 coloring, E133) from Vahiné (Avignon, France) was used as an aqueous colorant.  
119 Mesityl Oxide (4-methyl-4-penten-2-one) was purchased from Alfa Aesar (Thermo  
120 Fischer Scientific, Karlsruhe, Germany).

121

## 122 **2.2 Glaco™ coated capillaries**

123 Glaco Fused silica capillaries (PolyMicro Technologies, Photonlines, Saint-Germain-  
124 en-Laye, France) of 360 μm external diameter, 50, 100 or 180 μm internal diameter  
125 (I.D.) × 40 cm total length (30 cm to the detector window) were coated with Glaco™  
126 by flushing the capillary with the Glaco™ suspension. Excess of Glaco™ solution  
127 was then removed by flushing the capillary with air to obtain a 0.9 μm liquid film on  
128 the capillary wall, as given by equation (1) [37]:

$$129 \quad \varepsilon = 1.34 R_c^{7/3} \left( \frac{\Delta P}{8L\gamma} \right)^{2/3} \quad (1)$$

130 where  $\varepsilon$  is the thickness of the deposited film,  $R_c$  is the inner radius of the capillary,  
131  $\Delta P$  is the applied air pressure,  $L$  is the total capillary length and  $\gamma$  is the surface  
132 tension of the Glaco™ solution (20 N.m<sup>-1</sup>). Equation (1) was obtained by combining  
133 the Poiseuille with the Landau, Levich and Derjaguin (LLD) theory [37] giving:

$$134 \quad \varepsilon = 1.34 R_c Ca^{2/3} \quad (2)$$

135 where  $Ca$  is the dimensionless capillary number defined by equation (3):

$$136 \quad Ca = \frac{\eta V}{\gamma} \quad (3)$$

137 where  $V$  is the linear velocity of the coating solution flushed by air pressure (in m.s<sup>-1</sup>).  
138 The experimental conditions for the flushing pressures and times are displayed in  
139 Table 1 according to the capillary I.D. Glaco™ coatings were then thermally  
140 stabilized by placing the capillaries in a GC-2010 Plus oven (Shimadzu, Marne-la-  
141 Vallée, France) for 15 min at 250°C without any gas flow. The same process

142 (Glaco™ deposition and thermal stabilization) was repeated 3 times. Capillaries were  
143 finally left overnight under ambient air at room temperature before use.

144

145 **Table 1.** Coating protocol for the preparation of Glaco™ coated capillaries (Fused  
146 silica capillaries of 40 cm × 360 μm O.D. × I.D. as indicated in the Table). The entire  
147 protocol is repeated 4 times by alternating the flushing direction in the capillary  
148 (alternatively from the starting or the ending ends of the capillary).

149

Capillary I.D.	50 μm	100 μm	180 μm
Glaco™ flushing	20 psi, 5 min	5 psi, 5 min	1.5 psi 5 min
Air flushing	80 psi, 5 min	20 psi, 5 min	6.2 psi 5 min

150

### 151 **2.3 Capillary electrophoresis**

152 Capillary electrophoresis experiments were carried out on a P/ACE™ MDQ  
153 Beckman-Coulter (Sciex, Villebon sur Yvette, France) piloted by 32 Karat software.  
154 Glaco™ coated capillaries of 50 μm internal diameter (I.D.), 40 cm total length (30  
155 cm to the detector window), were coated as described in section 2.2 and used for air  
156 bubble generation. Unless otherwise specified, all electrolytes were prepared with  
157 boiled ultrapure water to obtain gas-free solutions. The degassing step appeared to  
158 be primordial in previous studies [36] as it allowed for stable current during the whole  
159 runs. Without degassing, the gasses dissolved into the aqueous phase interacted  
160 with the capillary air layer to form bubbles obstructing the flow and thus breaking the  
161 conductivity of the background electrolyte. Electrophoretic migrations were  
162 performed in a 20 mM HEPES + 10 mM NaOH background electrolyte at pH 7.4 (10  
163 mM ionic strength). Capillaries were flushed at 20 psi for 3 min with the background  
164 electrolyte before each run. DMF (at the concentration of 1 % in the electrolyte) was  
165 used as an electroosmotic flow marker. Electrophoretic migrations were realized  
166 using +20 kV voltage (unless otherwise specified) and +3 psi pressure at both  
167 capillary ends to stabilize the electrical current.

168

### 169 **3 Results and discussion**

170 Inadvertently and surprisingly, we have noticed that microbubbles could be easily  
171 generated in a controlled manner in 80 psi-Glaco™ coated capillaries. We have also  
172 noticed that these bubbles can move into the capillary and migrate under electric  
173 field. The aim of this study is to identify the conditions of appearance of these  
174 bubbles and to characterize these bubbles in terms of charge, electrophoretic  
175 mobility and size.

176

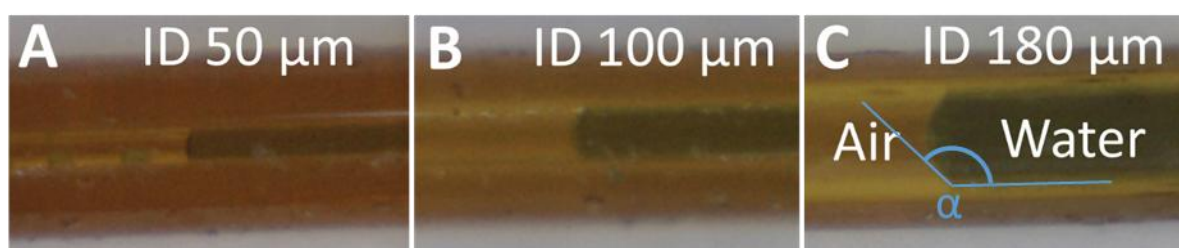
#### 177 **3.1 Highlighting the generation of air bubbles during** 178 **electrophoresis on a Glaco™ coated capillary**

179 Figure 1 shows the meniscus of a blue colored-water plug in a Glaco™ coated  
180 capillaries of different diameters (50, 100 and 180  $\mu\text{m}$ , respectively), according to the  
181 coating protocol detailed in section II.2 (with a deposition thickness of Glaco™  
182 solution of about 0.9  $\mu\text{m}$ ). The meniscus shape is reversed compared to fused silica  
183 capillary with a contact angle of about  $\alpha=120^\circ$ , which proves that the coating is  
184 highly hydrophobic but significantly lower than for a 1  $\mu\text{m}$  film deposition ( $\alpha=159^\circ$ )  
185 [36].

186 Figure 2 displays the current intensity (in green) and the UV trace (in blue) obtained  
187 in the Glaco™ coated capillary under +20 kV voltage in a 20 mM HEPES/Na buffer  
188 pH 7.4, when the inlet vial contains 1% DMF solution (used as EOF marker in frontal  
189 continuous electrophoresis mode) in the BGE. The increase of the UV trace  
190 corresponds to the detection of the DMF reaching the detection window.  
191 Surprisingly, a few seconds later, UV spikes appeared regularly with simultaneous  
192 fall of the current intensity. Spikes combined with current intensity drop can be  
193 explained by the formation of air bubbles generated at the detection point. The air  
194 bubble passing in front of the detection point modified the absorbance signal,  
195 generating a spike, and the presence of low-conductivity zone in the BGE decreased  
196 the current intensity. It is worth noting that these experiments were reproducible, as  
197 demonstrated by Figure SI1 showing similar results in the same electrophoretic  
198 conditions but on a different Glaco™ coated capillary according the same coating  
199 protocol.



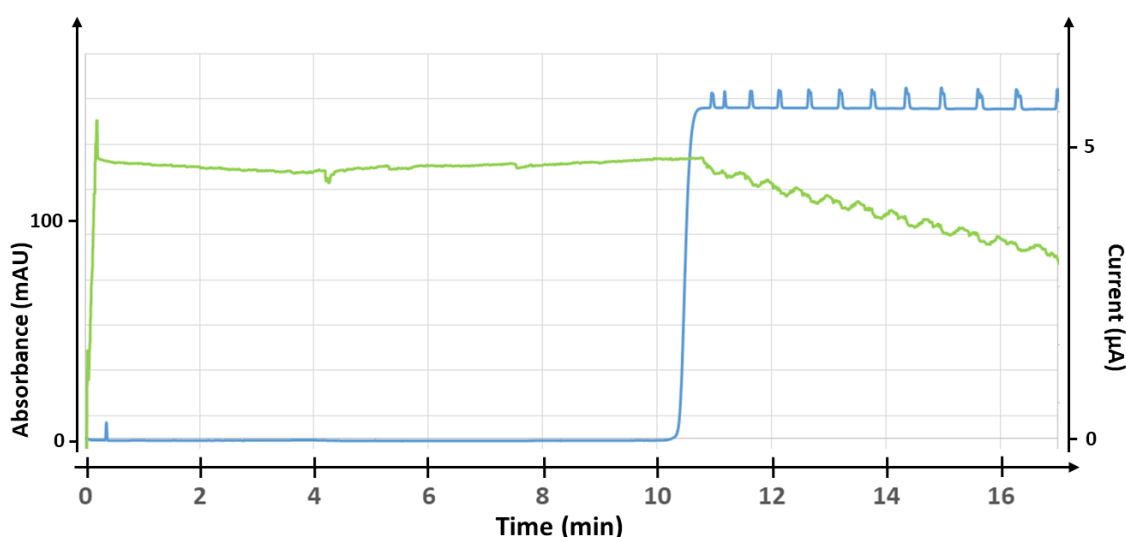
200 There are several experimental parameters that are required to generate the air  
201 bubbles. They are listed hereafter: (i) the 80 psi Glaco™ coated capillary is required;  
202 (ii) the UV lamp should be turned on since the electrical current intensity was not  
203 affected when the same experiment as in Figure 2 was conducted in absence of UV  
204 radiation; (iii) the separation voltage is also required and no bubble was observed  
205 under hydrodynamic flow without separation voltage; (iv) a DMF concentration of at  
206 least 0.5% is required (lower DMF concentrations or the use of other marker such as  
207 mesityl oxide did not generate bubbles, see Figure SI2).



208  
209 **Figure 1.** Visualization of the meniscus in Glaco™ coated capillaries of different  
210 internal diameters (**A:** 50 μm ID ; **B:** 100 μm ; **C:** 180 μm) by optical microscopy  
211 (magnification x50). Capillaries are filled with blue colored water (died with E133 at  
212 1 g.L<sup>-1</sup>) and with air to see the interface. Glaco™ coated capillaries were prepared  
213 according to the protocol given in section II.2. α is the contact angle (~120°).

214

215



216  
217 **Figure 2.** Electropherogram (blue trace) of 1% DMF marker in frontal mode obtained  
218 on Glaco™ coated capillary showing the generation of air bubbles. The  
219 corresponding current intensity is displayed in green. Experimental conditions:  
220 Glaco™ coated capillary 50 μm I.D. × 40 cm length (30 cm to the detector) prepared  
221 according to section 2.2. Background electrolyte: 20 mM HEPES + 10 mM NaOH at

222 pH 7.4. Applied voltage: +20 kV. Pressure (to stabilize the current intensity): + 3 psi  
223 at both capillary ends. Inlet vial: DMF at 1% in BGE. Outlet vial: BGE. Capillary flush  
224 with BGE before the run: 20 psi, 3 min. Detection: 214 nm. Temperature: 25°C.  
225

### 226 **3.2 Electrophoretic mobility and zeta potential of air bubbles**

227 Air bubble electrophoretic apparent velocity and dimensions can be both determined  
228 (or estimated) from the experiment described in Figure 3. Frontal continuous  
229 electrophoresis of 1% DMF in BGE was performed as in Figure 2 (see Figure 3, step  
230 1); however the voltage was rapidly stopped once a bubble was detected. Then, a  
231 mobilization pressure was applied in order to detect a second time the bubble in front  
232 of the detector (see Figure 3, step 3). The direction of the hydrodynamic flow to  
233 apply to detect the bubble a second time was identified on a trial and error principle,  
234 since it depends on the direction of the apparent velocity of the bubble during the  
235 electrophoretic process. It was found that the apparent electrophoretic velocity of the  
236 air bubble was against the electroosmotic flow (toward the inlet side of the capillary,  
237 as for a highly charged anion). Just before the mobilization pressure, the inlet vial  
238 was replaced by a 1.5% DMF in BGE (step 3) in order to be able to determine the  
239 mobilization pressure velocity.

240 In order to determine the apparent and effective electrophoretic mobility of the air  
241 bubble in the capillary during the electrophoretic process, it was assumed that the air  
242 bubble moved at the same velocity ( $v_{hydro,bubble}$ ) that the velocity of the DMF front  
243 ( $v_{hydro,DMF}$ ) during the mobilization pressure step (step 3 in Figure 3), leading to  
244 equation (4):

$$245 \quad v_{hydro,DMF} = \frac{l}{t_{hydro,DMF}} = v_{hydro,bubble} = \frac{d_{hydro,bubble}}{t_{hydro,bubble}} \quad (4)$$

246 where  $l$  is the effective capillary length,  $t_{hydro,DMF}$  is the migration time of the 1.5%  
247 DMF front to reach the detection window under 3 psi pressure (2.78 min) during the  
248 mobilization pressure step,  $d_{hydro,bubble}$  is the distance between the air bubble and the  
249 detection point just before applying the mobilizing pressure, and  $t_{hydro,bubble}$  is the time  
250 taken by the air bubble to reach the detection window once the pressure was applied  
251 (0.36 min). From equation (4), it is possible to determine  $v_{hydro,bubble} = 1.8 \times 10^{-3} \text{ m.s}^{-1}$   
252 and  $d_{hydro,bubble} = 38.8 \text{ mm}$ . Assuming that  $d_{hydro,bubble}$  is equal to the distance  
253  $d_{electro,bubble}$  travelled by the bubble from the detection point during the electrophoretic

254 process after the bubble formation, one can determine the apparent electrophoretic  
255 velocity  $v_{electro,bubble}$  of the air bubble during the electrophoretic migration by:

$$256 \quad v_{electro,bubble} = \frac{d_{hydro,bubble}}{t_{electro,bubble}} \quad (5)$$

257 where  $t_{electro,bubble}$  is the time between the air bubble first apparition at the detection  
258 window and the electrical field stop (0.34 min). From eq. (5), we get  
259  $v_{electro,bubble}=1.9 \times 10^{-3} \text{ m}\cdot\text{s}^{-1}$  corresponding to an apparent electrophoretic mobility of  
260  $\mu_{app} = -38.0 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ . Knowing that the electroosmotic mobility  $\mu_{eo} = +10.9 \times 10^{-9}$   
261  $\text{m}^2\text{V}^{-1}\text{s}^{-1}$  from the DMF migration time, the effective electrophoretic mobility of the air  
262 bubble was determined as:  $\mu_{ep} = \mu_{app} - \mu_{eo} = -48.9 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ . The air bubbles bear  
263 therefore strongly negative charges on their surfaces, which correspond to a zeta  
264 potential of about  $\sim -62.6 \text{ mV}$ , assuming that the air bubble behaves according to the  
265 Smoluchowski law:

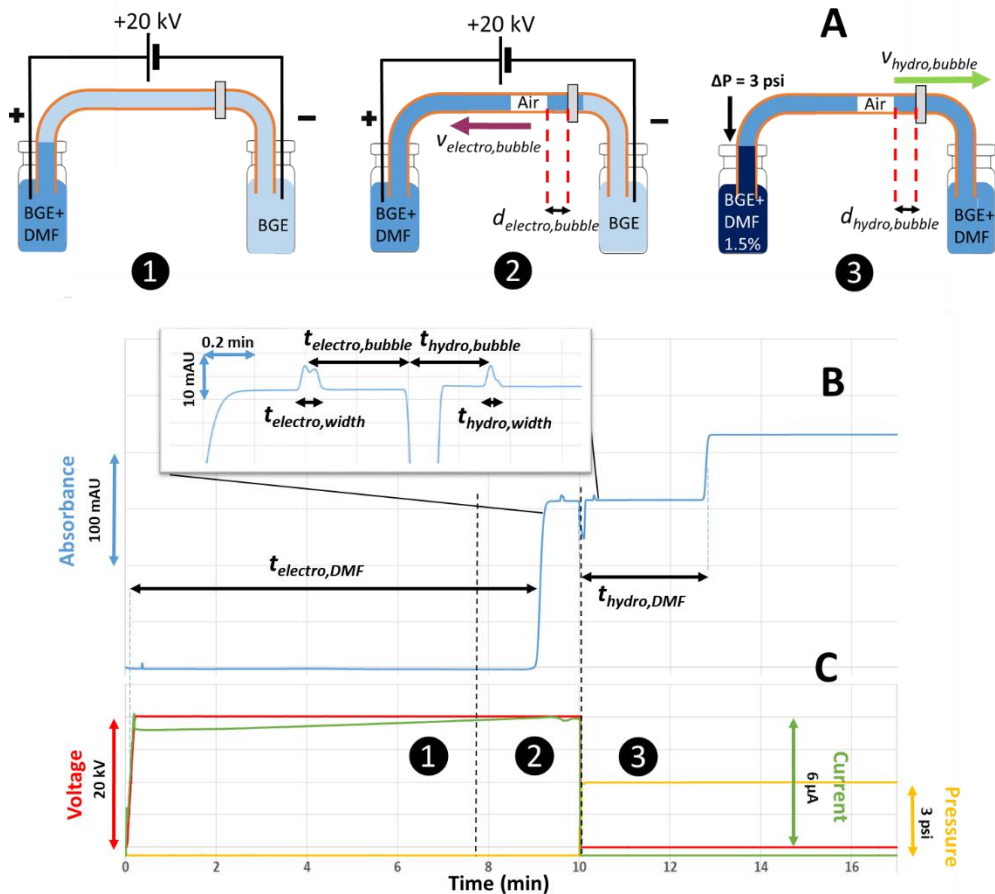
$$266 \quad \zeta = \frac{\mu_{ep} \eta}{\epsilon_0 \epsilon_r} \quad (6)$$

267 where  $\epsilon_0$  is the vacuum permittivity ( $8.85 \times 10^{-12} \text{ F}\cdot\text{m}^{-1}$ ),  $\epsilon_r$  is the water relative  
268 permittivity ( $\epsilon_r=78$ ), and  $\eta$  is the electrolyte viscosity (0.89 cP). The validity of the  
269 Smoluchowski law is verified since  $\xi < 100 \text{ mV}$  and  $\kappa R_h > 100$ , where  $\kappa$  is the  
270 reciprocal of the Debye length ( $\kappa^{-1} \sim 3 \text{ nm}$ ) and  $R_h$  is the characteristic size of the  
271 solute (air bubble in this study,  $\sim 40 \mu\text{m} \times 10 \text{ mm}$ , see section 3.3).

272 The origin of the negative charges at the liquid/gas interface can be attributed to the  
273 adsorption phenomena of  $\text{OH}^-$  anions on the bubble surface [33,34], which was also  
274 observed on solid nanoparticles [18]. This theory is supported by the fact that the  
275 bubbles were much more stable (i.e. the electrostatic force was higher) in alkaline  
276 water solution than in a neutral or acidic one. Some authors also considered the  
277 increased autolysis of water molecules to explain higher hydroxyde content at the  
278 surface [34]. But this general interpretation is still under debate, and other authors  
279 developed, more recently, a surface complexation model based on the presence of  
280 negative surface sites due to the fact that the balance of accepting and donating  
281 hydrogen bonds is broken at the interface [30,35]. Regarding the origin of the air/gas  
282 inside the bubble that we observed, since the BGE was degassed, a possibility could

283 be that some of the air trapped at the highly hydrophobic capillary surface is  
 284 destabilized and expelled in the bubbles.

285



286

287 **Figure 3.** Details of the experimental sequence implemented to determine the  
 288 dimensions and electrophoretic mobility of an air bubble generated during the  
 289 electrophoresis of a DMF front in a 80 psi-Glaco™ coated capillary. Schematic  
 290 representation of the DMF front and the air bubble positions in the capillary at  
 291 different times (A), of the UV trace at 200 nm (B) and the corresponding voltage (in  
 292 red) / pressure (in yellow) / current intensity (in green) sequence (C). Experimental  
 293 conditions: Glaco™ coated capillary, 50  $\mu\text{m}$   $\times$  40 cm length (30 cm to the detector).  
 294 Background electrolyte: 20 mM HEPES + 10 mM NaOH at pH 7.4. At  $t=0$ , BGE was  
 295 in the capillary and in the outlet vial, while the inlet vial contained BGE + DMF.  
 296 Separation voltage: +20 kV from 0 to 10 min. The 1% DMF front reached the  
 297 detection window at 9.1 min under electrophoretic process and generated one air  
 298 bubble detected at 9.7 min. The air bubble then migrated towards the inlet end of the  
 299 capillary until voltage was suppressed. From  $t=10$  min, the voltage was stopped,  
 300 the inlet vial was replaced by a vial containing 1.5% DMF in BGE and a + 3 psi  
 301 mobilization pressure was applied. At 10.4 min, the air bubble reached the detection  
 302 window, followed by the 1.5 % DMF front which reaches the detector at 12.8 min.  
 303 Flush before experiment: 20 psi, 3 min with BGE. Steps 1 to 3 are commented in the  
 304 text.

305

### 306 3.3 Air bubble dimensions

307 The dimensions of the air bubble can change under the influence of an electric field  
308 [38]. Knowing the apparent air bubble velocities both under electrical field  $v_{electro,bubble}$   
309 (equation 5) and under pressure  $v_{hydro,bubble}$  (equation 4), it was thus possible to  
310 calculate the air bubble length (or width) by measuring the time the air bubble took to  
311 cross the detection window (i.e. the temporal air bubble width measured as  $t_{electro,width}$   
312 = 0.092 min under electrical field and  $t_{hydro,width}$  = 0.067 min under pressure  
313 mobilization), using equations (7) and (8):

$$314 \quad l_{electro,bubble} = v_{electro,bubble} t_{electro,width} \quad (7)$$

$$315 \quad l_{hydro,bubble} = v_{hydro,bubble} t_{hydro,width} \quad (8)$$

316 Those equations lead to  $l_{electro,bubble} = 10.5$  mm and  $l_{hydro,bubble} = 7.24$  mm, illustrating  
317 the difference in size of a given air bubble with and without applied voltage,  
318 respectively. This is also in good agreement with the fact that the air bubble tends to  
319 spread under the influence of an electric field [38].

320 The presence of air bubbles in the capillary lowers the global conductivity of the  
321 capillary, since the air bubble are non-conductive. This explains why the current  
322 intensity is progressively decreasing with the generation of the air bubbles in the  
323 capillary. Since the current intensity is not completely shut down but just decreased,  
324 it can be considered that the air bubbles are not clogging all the capillary section  
325 (see Figure 4). The overall resistivity  $R$  of the capillary is the sum of the individual  
326 resistivity pertaining to each bubble and the resistivity of the portions of capillary with  
327 no air bubble. As a first approximation, we consider in this model that all the air  
328 bubbles have the same given length  $l_{electro,bubble}$ . Therefore, in an electrical point of  
329 view, the overall resistivity of the capillary of length  $L$  can be obtained by considering  
330 the resistivity  $R_{bubble}$  due to the  $n$  bubbles which occupy a length  $L_1$ , and the  
331 resistivity of the BGE on a capillary length  $(L-L_1)$ , as illustrated in Figure 4.  $R_{bubble}$  can  
332 be expressed as a function of  $L_1$ , the conductive section  $S_1$  in the air bubble zone,  
333 and the BGE conductivity  $\kappa$  according to:

$$334 \quad R_{bubble} = \frac{L_1}{\kappa S_1} \quad (9)$$

335 Similarly, the resistivity of the portion of capillary without air bubble is given by:

336 
$$R_{nobubble} = \frac{L-L_1}{\kappa S} \quad (10)$$

337 where  $S$  is the conductive section of the capillary without bubble and  $L$  the total  
 338 capillary length. The conductivity of the 20 mM HEPES/Na BGE is  $\kappa = 6.3 \cdot 10^{-2} \Omega^{-1}m^{-1}$   
 339 as calculated from the current intensity obtained in similar electrophoretic conditions  
 340 in a 50  $\mu m$  I.D.  $\times$  40 cm bare fused silica capillary (i.e. without any air inside the  
 341 capillary). In order to determine the air bubble diameter  $d_{air}$ , the Ohm law is  
 342 expressed using equations (9) and (10) by:

343 
$$V = RI = \left( \frac{L-L_1}{\kappa S} + \frac{L_1}{\kappa S_1} \right) I \quad (11)$$

344 Replacing  $L_1$  by  $n_{bubble} \times l_{electro,bubble}$  in equation (11) leads to equation (12) where  $1/I$  is  
 345 expressed by:

346 
$$\frac{1}{I} = \left( \frac{L - n_{bubble} l_{electro,bubble}}{S} + \frac{n_{bubble} l_{electro,bubble}}{S_1} \right) \frac{1}{\kappa V} \quad (12)$$

347 with  $n_{bubbles}$  being the number of bubbles present in the capillary at a given migration  
 348 time. Deriving equation (12) according to the migration time, leads to equations (13):

349 
$$\frac{d}{dt} \left( \frac{1}{I} \right) = \frac{dn_{bubble}}{dt} \frac{l_{electro,bubble}}{\kappa V} \left( \frac{1}{S_1} - \frac{1}{S} \right) \quad (13)$$

350 where  $d(1/I)/dt = 232.7 \text{ A}^{-1}\text{s}^{-1}$  is the slope of the dotted trace in Figure 5 and  
 351  $dn_{bubble}/dt = 0.0305 \text{ s}^{-1}$  is the slope of the orange trace in Figure 5. Equation (13) can  
 352 be used to determine  $S_1$  according to:

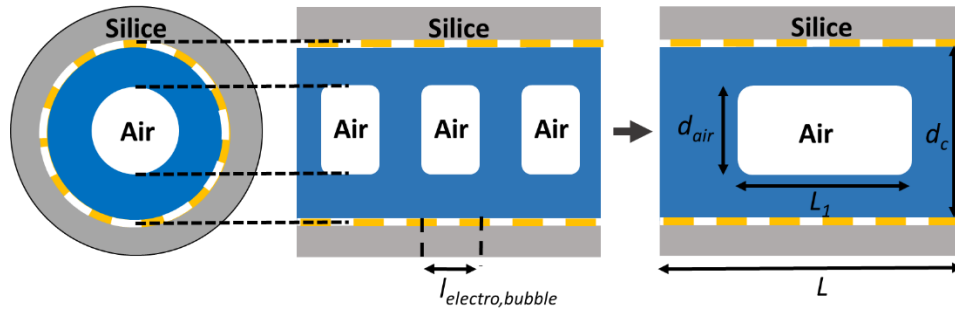
353 
$$S_1 = \frac{1}{\frac{\kappa V}{l_{electro,bubble}} \frac{d}{dt} \left( \frac{1}{I} \right) + \frac{1}{S}} \quad (14)$$

354 Taking  $l_{electro,bubble} = 13 \text{ mm}$  in equation (14) leads to  $S_1 = 7.99 \cdot 10^{-10} \text{ m}^2$ .  $d_{air}$  was then  
 355 determined using equation (15), with  $S_1$  obtained from equation (14), giving  $d_{air}$  of 37  
 356  $\mu m$ :

357 
$$d_{air} = \sqrt{\frac{4(S - S_1)}{\pi}} \quad (15)$$

358 Therefore, we can estimate the bubble diameter to  $\sim 37 \mu\text{m}$ . The experimental  
 359 method leading to equations (14) and (15) has the advantage to average the  
 360 experimental data on 11 bubbles created in a period of time corresponding to about  
 361 5-6 min (see Figure 5).

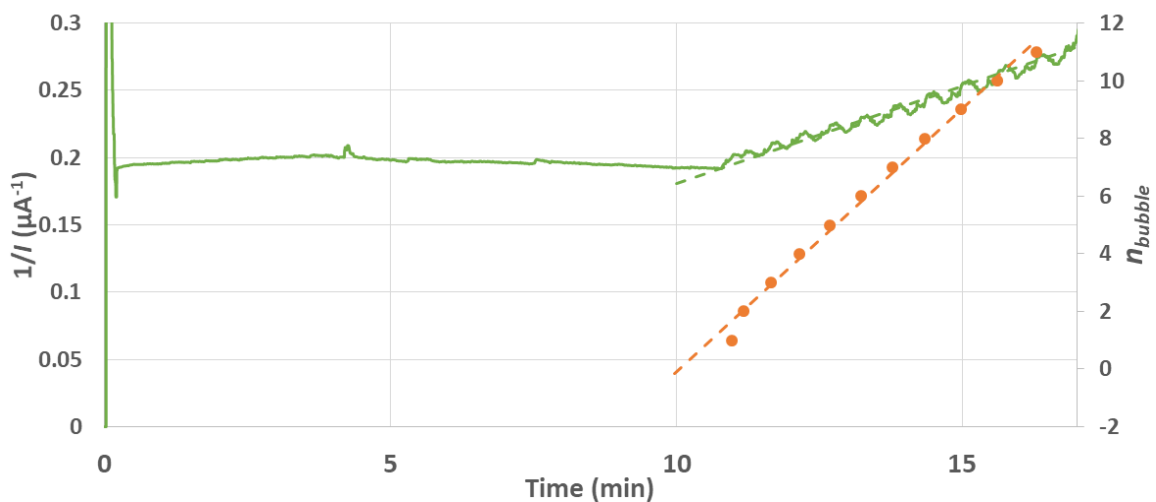
362



363

364 **Figure 4.** Schematic representation of the air bubble repartition in a Glaco™ coated  
 365 capillary for the modelling of the overall capillary resistivity  $R$ . The air bubbles are  
 366 considered as a succession of identical zones having a length  $l_{electro,bubble}$  under  
 367 electric voltage. In an electrical point of view, the capillary resistivity can be viewed  
 368 as the sum of two contributions: an air portion of capillary length  $L_1$  with a diameter  
 369  $d_{air}$  gathering all the air bubbles and a portion of capillary  $L-L_1$  only filled with BGE of  
 370 diameter  $d_c$ .  $S_1$  is the section of capillary occupied by the BGE in presence of an air  
 371 bubble and  $S$  is the section of capillary occupied by the BGE in absence of air  
 372 bubble.

373



374

375 **Figure 5.** Representation of the inverse of the current intensity evolution (green  
 376 trace) during DMF marker migration in a 80 psi-Glaco™ coated capillary and the  
 377 corresponding number of generated bubbles (in orange). Starting at around 11 min,  
 378 each current intensity perturbation increment is related to the formation of one air  
 379 bubble (also identified by an orange dot). The global decreasing current intensity  
 380 slope is  $d(1/I)/dt = 232.7 \text{ A}^{-1}\text{s}^{-1}$ . The slope  $dn_{bubble}/dt = 1.83 \text{ bubble min}^{-1} = 0.0305$

381 bubble  $s^{-1}$  (slope of the dotted orange line). Experimental conditions and  
382 experimental data as in Figure 2.

383

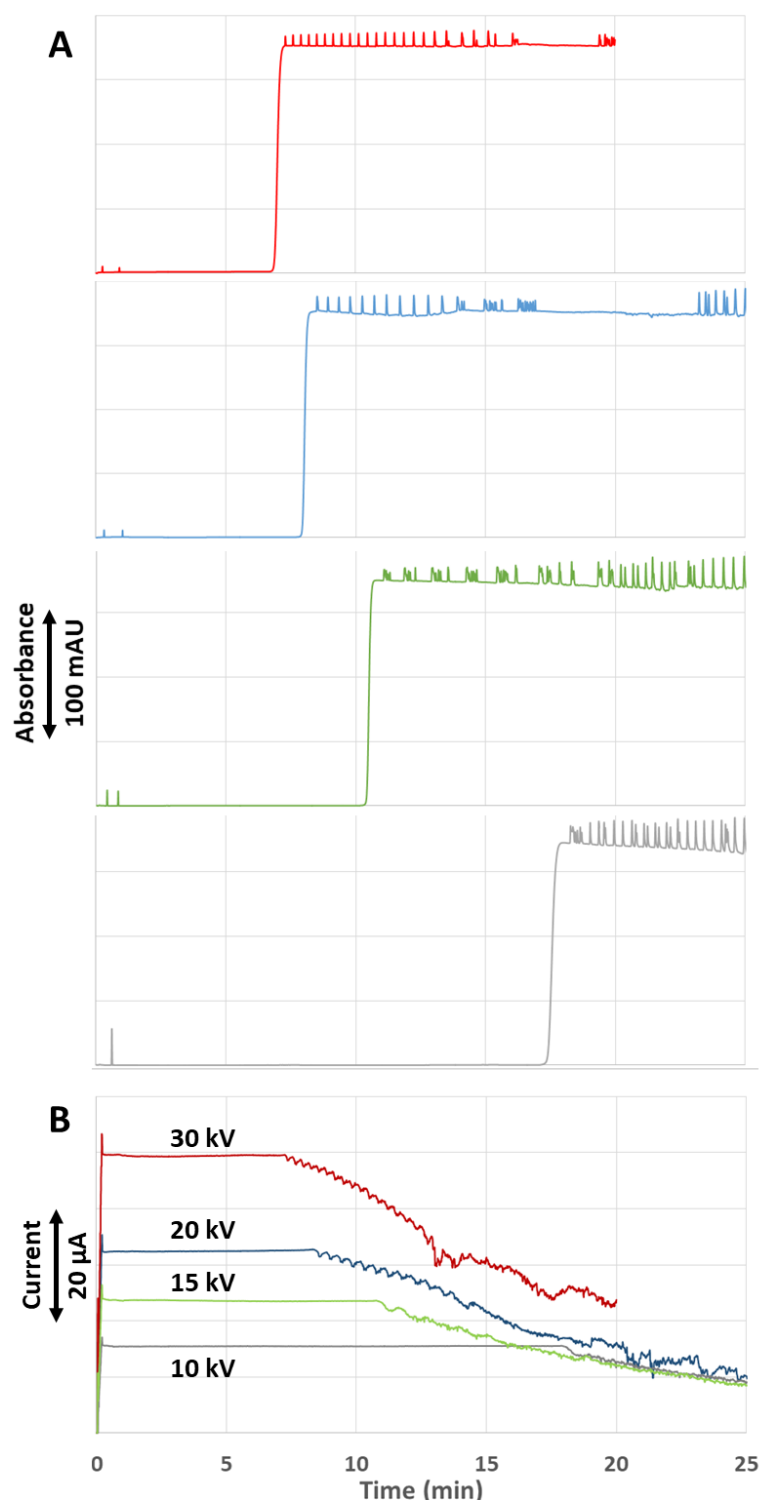
### 384 **3.4 Influence of the applied separation voltage on the bubble** 385 **formation and characteristics**

386 DMF migration similar to the experiment of Figure 2 was performed at 4 different  
387 voltages (30 kV, 20 kV, 15 kV and 10 kV) as displayed in Figure 6 showing the  
388 corresponding UV traces and current intensities. The bubble characteristics  
389 ( $I_{electro,bubble}$ ,  $d_{air}$ , bubble frequency, distance between bubbles) and the  
390 electrophoretic figures of merit (EOF mobility, electrophoretic velocity of the bubble)  
391 have been studied. The measurements were made on at least 6 successive bubbles  
392 on each run, and the results are summarized in Table 2. Calculations were done  
393 following the same approach as used in section 3.3. As expected, modifying the  
394 separation voltage allowed decreasing the apparent electrophoretic velocity value  
395 from  $2.95 \text{ mm}\cdot\text{s}^{-1}$  at 30 kV down to  $0.94 \text{ mm}\cdot\text{s}^{-1}$  at 10 kV. It is thus possible to  
396 modulate the migration velocity of the bubbles, by simply changing the applied  
397 voltage. Regarding the dimensions of the bubbles, the diameter of the bubble was  
398 relatively constant (about  $d_{air} \sim 36\text{-}41 \mu\text{m}$ ), whatever the applied voltage. The bubble  
399 length under electric field was about  $l_{electro,bubble} \sim 8\text{-}9 \text{ mm}$ , except at 10 kV for which  
400 the length was about 2 times lower ( $\sim 4.4 \text{ mm}$ ). The frequency of the bubbles was  
401 comprised between 0.053 Hz and 0.035 Hz.

402  $\mu_{eo}$  was roughly the same at each applied voltage, as expected. Hence,  $V_{electro,bubble}$   
403 was directly proportional to the applied voltage. The distance between two bubbles is  
404 the same at 30 kV and 20 kV (about 5 cm), and decreased to about 2 to 3 cm at 10  
405 kV and 15 kV, respectively. On the whole, better stability and regularity in the bubble  
406 formation was observed at 30 kV and 20 kV compared to 15 and 10 kV.

407





408

409 **Figure 6:** Electropherograms of 1% DMF marker in frontal mode obtained on 80 psi-  
 410 Glaco™ coated capillary showing the generation of air bubbles at 30 kV (light red),  
 411 20 kV (light blue), light green (15 kV) and 10 kV (light grey) (A) and the  
 412 corresponding current intensities (same color but darker) (B). Experimental  
 413 conditions: Glaco™ coated capillary 50  $\mu$ m I.D.  $\times$  40 cm length (30 cm to the  
 414 detector) prepared according to section II.2. Background electrolyte: 20 mM HEPES  
 415 + 10 mM NaOH at pH 7.4. Pressure (to stabilize the current intensity): + 3 psi at both  
 416 capillary ends. Inlet vial: DMF at 1% in BGE. Outlet vial: BGE. Capillary flush with  
 417 BGE before the run: 20 psi, 3 min. Detection: 214 nm. Temperature: 25°C.

418

419 **Table 2.** Bubble characteristics at three different applied voltages. Experimental  
 420 conditions as in Figure 6. Electrophoretic mobility of the bubble:  $\mu_{ep} = -48.9 \times 10^{-9}$   
 421  $\text{m}^2\text{V}^{-1}\text{s}^{-1}$ .

Voltage (kV)	30	20	15	10
$\mu_{eo} (10^{-9} \text{m}^2 \text{V}^{-1} \text{s}^{-1})$	9.5	12.4	12.7	11.4
$\mu_{app} (10^{-9} \text{m}^2 \text{V}^{-1} \text{s}^{-1})$	-39.4	-36.4	-36.2	-37.5
$v_{electro,bubble} (\text{mm} \cdot \text{s}^{-1})$	2.95	1.82	1.36	0.94
$l_{electro,bubble} (\text{mm})^a$	8.7	9.3	8.5	4.6
Bubble frequency ( $\text{s}^{-1}$ )	0.0527	0.0345	0.0401	0.0516
$d_{air} (\mu\text{m})$	35.8	35.9	41.2	39.0
Distance between bubbles (mm)	55.9	52.7	33.8	18.2

422 <sup>a)</sup> mean value of the first 11 bubble lengths of each run for the runs at 30 and 20 kV, and mean value  
 423 of the 6 more stable bubbles for the run at 15 kV (the six consecutive bubbles between 24 and 26  
 424 min) and 10 kV (the six consecutive bubbles between 19 and 21 min).

425

## 426 **4 Concluding remarks**

427 This work demonstrates that it is possible to repeatedly generate air microbubbles at  
 428 the detection window of an hydrophobic Glaco<sup>TM</sup> coated capillary. In addition to the  
 429 specific capillary coating, several experimental requirements have been identified: (i)  
 430 the UV lamp should be turned on; (ii) the separation voltage is also mandatory; (iii) a  
 431 DMF concentration of at least 0.5% is required in front of the detection window to  
 432 generate the bubbles. Air microbubbles have a diameter of about  $d_{air} \sim 35\text{-}39 \mu\text{m}$ , a  
 433 typical length of about 10 mm and an effective electrophoretic mobility of about -  
 434  $48.9 \times 10^{-9} \text{m}^2\text{V}^{-1}\text{s}^{-1}$ . The air bubbles bear therefore strongly negative charges on their  
 435 surfaces corresponding to a zeta potential of about  $\sim -62.6 \text{mV}$ . As expected,  
 436 modifying the separation voltage allows changing the apparent electrophoretic  
 437 velocity from  $2.95 \text{mm} \cdot \text{s}^{-1}$  at 30 kV down to  $0.94 \text{mm} \cdot \text{s}^{-1}$  at 10 kV. This approach  
 438 could be useful to generate on demand air microbubbles, for instance by controlling  
 439 the zones of DMF in the capillary, or by controlling the switching on/off of the UV  
 440 beam during the run.

441

## 442 **Conflict of interest**

443 The authors have declared no conflict of interest.

444

## 445 **Data Availability Statement**

446 The data that support the findings of this study are available from the corresponding  
447 author upon reasonable request.

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512

## 513 **Supporting information**

514 **Supporting information file:** This Supporting information includes:

515 SI-1. Bubble generation reproducibility

516 SI-2. Required parameters for bubble generation: influence of the DMF concentration  
517 and nature of the neutral marker

518

519