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1 **Electroosmotic flow modulation for improved electrokinetic preconcentration :**
2 **application to capillary electrophoresis of fluorescent magnetic nanoparticles**

3
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16 **Keywords:** capillary electrophoresis; fluorescence detection; stacking; preconcentration; EOF
17 suppression and modulation; magnetic nanoparticles

18

19 **Abstract**

20 It is reported in this study a new approach for modulation and even suppression of the electroosmotic
21 flow (EOF) to achieve better electrokinetic preconcentration in capillary electrophoresis This is
22 based on the augmentation of the buffer's concentrations to very high levels (more than a thousand
23 of mM) without recourse to any dynamic/permanent coating nor viscous gel. The use of large weakly
24 charged molecules as background electrolyte's constituents allows working at extreme concentration
25 ranges without penalty of high electric currents and Joule heating. By this way, the electroosmotic
26 mobility could be modulated over a wide range ($2 - 60 \times 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ under alkaline conditions),
27 and suppressed to levels equivalent to those obtained with several neutral coatings. For
28 demonstration, this new approach was applied for sensitive determination of core-shell magnetic
29 nanoparticles (CSMNPs) having high potential for healthcare applications such as imaging agents for
30 diagnostics and controllable cargos for nanomedicine. Different profiles were achieved for purpose-
31 made and commercial magnetic nanoparticles using CE coupled with light-emitting-diode induced
32 fluorescence (LEDIF) detection. Compared to the conventional capillary electrophoresis (CE-UV)
33 method for characterization of magnetic nanoparticles, our proposed approach with fluorescent
34 detection and EOF-assisted preconcentration offers almost 350-fold sensitivity improvement.
35 Furthermore, our scheme can be used for monitoring the interaction between CSMNPs and target
36 pharmaceutical molecules, serving for drug delivery development. A preliminary study with two
37 antibiotics using this approach revealed that kanamycin interacts better with the target nanoparticles
38 than amikacin.

39

40

41

42 **1) Introduction**

43 Nanoparticles are appealing candidates for biomedical and analytical applications (e.g. imaging, drug
44 delivery, sensors, magneto-immunoassays, lab on chip development). Their analysis is essential to
45 obtain information on their size, shape, potential heterogeneity and surface chemistry or
46 functionalization, enabling their practical use for various applications [1, 2]. Capillary electrophoresis
47 (CE) is a well-described technique to characterize nanoparticles and monitor their interaction with
48 target (pharmaceutical and biological) molecules [3]. These CE characterization methods are so far
49 coupled with either UV or MS detection [2, 4]. CE with laser-induced fluorescent (LIF) detection of
50 nanoparticles (other than quantum dots) has never been communicated to the best of our knowledge.
51 As detection sensitivity is always limited with reported CE-UV or CE-MS approaches, some specific
52 modes of on-line preconcentration are often needed prior to electrophoretic separations of
53 nanoparticles [5]. In the case of core-shell magnetic nanoparticles (CSMNPs) that have been
54 exploited in different health-care applications (i.e. multimodal imaging for diagnostics and
55 controllable cargo for drug delivery [6-11]), two approaches for on-line electrokinetic
56 preconcentration prior to their CE-UV separations have been communicated so far. These include
57 field-amplified sample injection [12, 13] and dynamic pH junction [14], allowing preconcentration
58 of sample plugs accounting for less than 30 % of the total capillary volume. Due to the heterogeneous
59 population of nanoparticles in general, and CSMNPs in particular, electrokinetic preconcentration
60 from a more significant sample volume (*i.e.* equivalent to 100 % of the capillary one) is more
61 challenging and has not been explored so far.

62
63 Large volume sample stacking (LVSS) with polarity switching is a performing electrokinetic
64 preconcentration mode in CE that exploits the bulk electro-osmotic flow (EOF) to allow stacking of

65 target analytes for enrichment [15-24]. Preconcentration in this case can be realized even from a
66 sample solution filling up to 100 % of the capillary volume. LVSS with polarity switching in fused
67 silica capillaries requires however two different EOF magnitudes for sample preconcentration and
68 then separation of stacked analytes. A high EOF is needed to remove sample matrix from the capillary
69 during the stacking of negatively charged analytes whereas a much lower one (even suppressed EOF)
70 is often required to ensure baseline separation of enriched species. This EOF change needed for LVSS
71 with polarity switching can be created through significant buffer's pH variation or the use of neutral
72 capillary coatings [15-24]. To reduce EOF under neutral and alkaline conditions, dynamic and
73 permanent capillary coatings are commonly employed [25, 26]. An alternative to conventional
74 capillary coating methods has been recently developed, exploiting the potential of nanomaterials as
75 nano-coating layers [27]. EOF can also be suppressed by significantly increasing the electrolyte
76 viscosity [28, 29]. Accordingly, viscous gels (i.e. polyethylene oxide gel and acrylamide-based
77 hydrogel) were employed to block the apparent electro-osmotic (EO) fluid flow [30, 31]. The increase
78 in buffer viscosity to reduce EOF can also be carried out by addition of organic modifiers (glycerol,
79 chemical gels or polymer solutions) to the running buffer [32]. Another option is to increase the
80 electrolyte's ionic strength (IS) to such a high level that sufficiently compresses the thickness of the
81 diffusion double layer [33-39]. Nevertheless, this approach normally comes with high current
82 generation and unwanted Joule heating effect with conventional CE electrolytes containing inorganic
83 ions.

84
85 Herein, we report for the first time a CE coupled with light-emitting-diode (LED) induced
86 fluorescence detection approach (CE-LEDIF) for CSMNPs, offering significant improvement of
87 detection sensitivity compared to the previously reported methods using UV or inductively coupled

88 plasma mass spectrometry (ICP-MS) detection [40-43]. This was achieved with a novel way to
89 perform on-line electrokinetic preconcentration of CSMNPs via modulation of the EO mobility (μ_{EO})
90 in fused silica capillaries without recourse to any coating nor viscous gel. This method for EOF
91 modulation is based on the augmentation of the buffer's concentrations to very high levels (more than
92 a thousand of mM) using selected background electrolytes (BGEs) composed of large, weakly
93 charged molecules. The magnitude of EOF could be regulated or suppressed whatever the pH (neutral
94 and alkaline ranges) of the BGE without incurring high electric currents. The coupling of our coating-
95 free electrokinetic preconcentration and CE-LEDIF analysis for sensitive detection of CSMNPs as
96 well as monitoring of their interaction with different target pharmaceutical compounds is presented.

97

98 **2. Experimental**

99 ***2.1. Chemicals and reagents***

100 2-(Cyclohexylamino)ethanesulfonic acid (CHES), 3-(Cyclohexylamino)-1-propanesulfonic acid
101 (CAPS), 2-(N-morpholino)ethanesulfonic acid (MES), phosphoric acid, 3-(N-
102 morpholino)propanesulfonic acid (MOPS), Triethanolamine (TEA, $\geq 98\%$), Diethanolamine (DEA,
103 $\geq 98\%$), Ethanolamine ($\geq 98\%$), Tris(hydroxymethyl)aminomethane (TRIS), sodium dodecyl
104 sulphate (SDS), sodium hydroxide (NaOH), acid boric (H_3BO_3), sodium dihydrophosphat
105 (NaH_2PO_4), disodium hydrophosphat (Na_2HPO_4), Dimethyl sulfoxide (DMSO 99.9%), amikacin
106 sulfate were all provided by Sigma (St. Louis, MO, United States). Kanamycin sulfate was obtained
107 from Sequoia Research Product Ltd (Pangbourne, UK). Fluorescein isothiocyanate (FITC) was
108 purchased from Beckman (Sciex Separation, Brea, CA). All buffers were prepared with deionized
109 water and were filtered through a 0.22 μm membranes (Pall Corporation, New York, USA) prior to

110 use. Carboxylate-modified nanoparticles, fluorescent ($\lambda_{\text{ex}} \sim 470$ nm; $\lambda_{\text{em}} \sim 505$ nm), 30 nm in
111 diameter were obtained from Sigma (St. Louis, MO, United States).

112

113 **2.2. Apparatus and Material**

114 Uncoated capillaries were purchased from Phymep (Paris, France). All experiments were performed
115 using a Beckman Coulter PA 800 plus system (Sciex Separation, Brea, CA) equipped with UV
116 detection and Agilent CE G1600AX Capillary Electrophoresis System (California, USA) coupled
117 with a LED-based fluorescence detector (LEDIF with $\lambda_{\text{excitation}}$: 480 nm, $\lambda_{\text{emission}}$: 520 nm, Adelis,
118 Labege, France). Data acquisition for UV detection and instrument control were carried out using
119 Karat 9.1 software (Sciex Separation, Brea, CA) whereas fluorescence detection data were recorded
120 by Power Chrome software (eDAQ, Australia). Deionized water used in all experiments was purified
121 using a Direct-Q3 UV purification system (Millipore, Milford, MA, USA). Conductivity and pH
122 values of buffer solutions and samples were measured by a Seven Compact pH meter (Mettler Toledo,
123 Schwerzenbach, Switzerland). Selection of background electrolyte (BGE) and buffer IS calculations
124 were simulated by the computer program PhoeBus (Analis, Suarlée, Belgium).

125

126 **2.3. Methods**

127 *Synthesis and physio-chemical characterization of core-shell MNPs*

128 The $\text{Fe}_2\text{O}_3@ \text{SiO}_2\text{-COOH}$ core-shell MNPs were synthesized according to reported protocols [9, 44].
129 Maghemite MNPs ($\gamma\text{-Fe}_2\text{O}_3$, with a mean diameter of 12.3 nm) were prepared following the
130 procedure described by Massart [45]. They were subsequently size-sorted in order to have a narrower
131 size distribution and then coated with citrate anions [46]. The obtained solution (121.2 μL) of citrated
132 maghemite MNPs at an iron concentration of 1.9 mol/L was dispersed in 10 mL of water and 20 mL

133 of ethanol. A coating by a fluorescent silica shell was performed by adding 152.6 μL of
134 tetraethylorthosilicate (TEOS, Merck), 500 μL of a 30% ammonia solution, and 25.4 μL of
135 aminopropyltriethoxysilane (APTS)-functionalized fluorescein (Sigma-Aldrich) at 18.75 mmol/L.
136 After 2 h of agitation, the surface of the silica shell was functionalized with amino groups and short
137 polyethyleneglycol (PEG) chains by the simultaneous condensation of APTS ($V = 27 \mu\text{L}$) and a silica
138 PEG-derived compound, 2-(methoxy(polyethyleneoxy)propyl)trimethoxysilane (PEOS, ABCR) (V
139 $= 61.8 \mu\text{L}$), with an appropriate amount of TEOS ($V = 50.8 \mu\text{L}$) in order to generate a cross-linked
140 silica shell. The mixture was stirred overnight. The resulting nanoparticles were then rinsed 3 times
141 with a mixture of diethylether/ethanol 15:1 and finally re-dispersed in 5 mL of a 3-
142 morpholinopropane-1-sulfonic acid (MOPS) buffer at 0.1 mol/L and $\text{pH} = 7.4$. These $\text{Fe}_2\text{O}_3@ \text{SiO}_2$ -
143 NH_2 MNPs were then converted to $\text{Fe}_2\text{O}_3@ \text{SiO}_2$ -COOH MNPs via a 45 min reaction of 3 mL of this
144 dispersion of MNPs with 1 mL of a solution of succinic anhydride at 0.5 mol/L in DMSO. Finally,
145 the $\text{Fe}_2\text{O}_3@ \text{SiO}_2$ -COOH MNPs were washed with MOPS buffer in PD-10 columns containing
146 Sephadex G-25 (GE Healthcare).

147
148 The $\text{Fe}_2\text{O}_3@ \text{SiO}_2$ -COOH MNPs were then characterized by transmission electron microscopy (TEM)
149 on a JEOL 1011 instrument, and by dynamic light scattering (DLS) and zetametry with a Zetasizer
150 Nano ZS (Malvern Instruments). The mean physical diameter of the MNPs was measured to be 31.7
151 $\pm 4.4 \text{ nm}$ (see Figure S1 in the electronic supplementary information ESI). The zeta potential and
152 hydrodynamic diameter of the MNPs in MOPS buffer were measured to be $-23 \pm 4.4 \text{ mV}$ and 32 nm
153 respectively (see Fig. S1 in the ESI) with a polydispersity index of 0.25.

154

155 *Measurement of electroosmotic flow (EOF)*

156 EOF was measured using the Beckman Coulter system and the signals were detected with UV
157 detection at 200 nm. The fused silica capillary (I.D. of 50 μm , effective length (L_{eff}) of 10 cm and
158 total length (L_{tot}) of 40 cm) was pre-conditioned with NaOH 1 M for 5 min, DI water for 5 min and
159 BGE for 15 min prior to the first use and post-conditioned with BGE for 3 min after each analysis.
160 The EOF marker, DMSO (1 % in water) was injected for 10 sec at 50 mbar, followed by application
161 of a voltage of 17 kV. All EOF measurements were performed in triplicate. Between analysis, the
162 capillary was rinsed with NaOH 1 M for 3 min, DI water for 2 min and BGE for 5 min.

163

164 *CE-LEDIF of FITC and core-shell MNPs*

165 These experiments were carried out with the Agilent system coupled with LIF and UV detection.
166 Prior to the first use, the silica capillary (I.D. of 50 μm , L_{eff} of 50 cm for UV detection, L_{eff} of 35 cm
167 for LIF detection and L_{tot} of 60 cm) was flushed with following sequence: NaOH 1 M for 5 min, DI
168 water for 5 min, then BGE for 25 min. Before each analysis, the capillary was flushed with SDS for
169 5 min, NaOH for 5 min, then DI water for 3 min and BGE for 5 min. FITC or CSMNPs samples were
170 hydrodynamically injected at 50 mbar for 30 s. FITC samples for CE-LEDIF were prepared by
171 dilution of the stock solution FITC (1000 nM) with deionized water whereas CSMNPs samples were
172 prepared by dilution of the stock $\text{Fe}_2\text{O}_3@\text{SiO}_2\text{-COOH}$ (whose iron concentration was 0.024 M) with
173 Tris/ CHES (IS 10 mM, pH 8.5). The samples were prepared daily. The separation was performed
174 under 25 kV (normal polarity) at 25 $^\circ\text{C}$. The signal of EOF was recorded by UV detection whereas
175 FITC and MNPs signals were observed by LIF detection. The fluorescent signals (analog signals)
176 were recorded with a data acquisition system and converted into digital ones, which are displayed in
177 the mV scale.

178

179 *Large volume sample stacking (LVSS) with polarity switching - CZE*

180 For LVSS of FITC or CSMNPs, samples were filled to 100 % of the capillary volume. A voltage of
181 - 25 kV was applied at the injection end (inlet) of the capillary for 5 min. The current was monitored
182 visually during this process. When the current reached the stable value after a stacking duration of 5
183 min, the polarity of the high voltage was immediately switched to trigger the separation. The
184 separation was conducted for 30 min under 25 kV with normal polarity.

185

186 **3. Results and Discussion**

187 **3.1. EOF-assisted preconcentration in an uncoated fused silica capillary**

188 *3.1.1. Suppression and modulation of EO mobility via variation of BGE concentrations*

189 Our coating-free approach to significantly reduce EOF under alkaline conditions in fused silica
190 capillaries is based on the use of BGEs at extremely high concentrations. This approach so far is not
191 trivial with conventional BGEs for CE-UV and CE-LIF, which normally contain inorganic ions for
192 buffering and pH adjustment, due to too high current generation at certain ionic strength (IS) ranges.
193 In our study, various BGEs composed of large weakly charged ions were investigated regarding their
194 ability to prevent Joule heating at high IS values. Among all BGEs simulated with the Phoebus
195 software (table 1), we selected 16 compositions covering the pH range from 5.5 to 9.6 for further
196 experiments on μ_{EO} suppression. For a selected anion and at the predefined IS of 150 mM, the cations
197 that allow to produce the highest simulated concentrations and the lowest simulated currents were
198 chosen. Accordingly, four organic acids, including CAPS, CHES, MOPS and MES were combined
199 with four organic bases (triethanolamine, diethanolamine, ethanolamine and Tris). Working with
200 such BGEs at this IS range (150 mM and more) is considered extreme because the constituent
201 concentrations can go up to a thousand mM or more (see table 1), which are very high compared to

202 those in conventional buffers for CE. The capability for EOF suppression of these BGE compositions
203 is reflected by the measured μ_{EO} values (Fig. 1). Some μ_{EO} measurements were also carried out with
204 the method of Williams and Vigh [47]. The results obtained (table S1 in the ESI) showed similar
205 ranges with no remarkable systematic bias between two different approaches for μ_{EO} measurements.
206 We can assume that the deviation linked to the non-thermostated section of the capillary encountered
207 in the short-end injection method for μ_{EO} measurement is negligible. Whatever the pH and tested
208 combination, the EOF was found to decrease drastically from more than $40 \cdot 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ (*i.e.* the
209 normal EOF magnitudes observed with conventional BGEs for CE at these pH values) to less than
210 $10 \cdot 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$. In some cases, EOF dropped down to $2 \cdot 10^{-5} \text{ (cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1})$, which is a μ_{EO} value
211 achieved with different home-made coatings [48, 49] and is very close to that obtained with a
212 commercial permanent neutral one (poly-vinyl alcohol (PVA) coating, $0.5 - 1 \cdot 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$) [50].
213 This is the first time very low μ_{EO} values, close to total EOF suppression, have been achieved with
214 such a principle. Furthermore, the desired range of EOF can be easily tuned just by changing the
215 BGE's concentrations. This is on the other hand not trivial with coated capillaries which generally
216 exhibit fixed μ_{EO} values. Capillary regeneration which is sometimes limited by chemical stability of
217 the coatings is now possible with our IS-based approach. Among all tested cations, the most efficient
218 for EOF reduction was triethanolamine, followed by diethanolamine (see Fig. 1). Besides the
219 contribution of extremely high BGE concentrations (to provide elevated IS values), alkylamines as
220 the cationic BGE constituents are known to reduce the EOF, which explains the excellent efficiency
221 in EOF blocking offered by the tested BGEs. Note that the current was measured over the whole
222 capillary, rather than for only the non-thermostated length (10 cm). In both measurements a bias due
223 to the non-cooled capillary section may occur [51]. Nevertheless, we considered this bias negligible,
224 as the surrounding temperature was less than 30°C [52]. In addition, the relatively small currents

225 generated with our BGEs ensured the Joule heating to be reduced, which in turn help minimize the
226 bias due to the non thermostated part of the capillary. The Ohm's law is strictly fulfilled at electrical
227 fields less than 250-300 V/cm for all tested buffers (see Fig. S2 in the ESI). Depending on the
228 composition of the BGEs, the linear relationship between voltage vs. generated current can be
229 maintained up to 400 V/cm in some cases (for instance the BGE composed of TEA-CHES with ionic
230 strength up to 240 mM). The currents measured for all these BGEs, even at their highest IS values,
231 remain inferior to 50 μ A under an electrical field of 400 V/cm. This is considered tolerable for a
232 capillary of 50 μ m ID [53]. In our IS-based approach for EOF suppression, the increase in IS of the
233 BGE is not limited by the generated current as in conventional buffers for CE, but rather by the
234 solubility of the least soluble component of the BGE. The highest BGE concentrations (or IS values),
235 and the lowest EOF magnitudes, accordingly, were achieved with diethanolamine/CAPS (250 mM,
236 pH 9.5), Tris/CHES (280 mM, pH 8.7) and triethanolamine/CHES (250 mM, pH 8.5). It is possible
237 that the amine moiety of the tested anions interacts with the silanols and contributes to some certain
238 extend to diminish the EOF. However, we expect this effect to be negligible compared to that
239 provoked by the cationic constituent of the buffer. The total net charge of the tested anions in the
240 working pH ranges is negative, meaning that the positive charge of its amine moiety is outnumbered
241 by the sulfonic acid negative one. The anions have therefore less chance than the cations to approach
242 the inner capillary surface; and the electrostatic interaction between the anions' amine moiety with
243 silanols is expected to be much less favorable.

244
245 In our approach for EOF suppression, we considered simultaneously different parameters (*i.e.* BGE's
246 IS, concentrations and generated electric currents) rather than focusing only on the key factor IS as
247 in previous studies [33-39]. It is well known that an increase in the buffer's IS can lead to reduction

248 of the electrical double layer thickness, thus decreasing EO mobility. However, for the same IS,
 249 different BGEs provided different EOF magnitudes, as demonstrated clearly in Fig. 1. Accordingly,
 250 EOF is not only dependent on the IS of the BGE, but also on its constituents' nature and
 251 concentrations which include the cations that can interact with the silanols [54]. Effort was therefore
 252 made to establish a theoretical index of a good BGE in terms of EOF reduction capacity, based on
 253 the simulated parameters and the measured μ_{EO} values (Fig. 2). A good buffer for EOF suppression
 254 should possess an IS and constituents' concentrations as high as possible while generating an electric
 255 current (I) as low as possible. The nature and concentration of cations in the BGE significantly
 256 contribute to the μ_{EO} . The cation's concentration therefore is expected to play a more pronounced role
 257 for EOF suppression. A high buffer capacity is also another factor to consider for a good BGE.
 258 Accordingly, we propose a good-buffer index (GBI) as follow:

$$259 \quad GBI = \frac{IS \times (C_C + C_A) \times C_C \times \beta}{I}$$

260 *with C_A , C_C (mM) as the concentrations of the BGE constituents (e.g. for Tris / CHES BGE, $C_A =$*
 261 *C_{CHES} and $C_C = C_{Tris}$); β (mM/pH) as the buffer capacity; and I (μA) as the measured electric current*
 262 *generated by the BGE under a fixed electrical field of 400 V/cm.*

263 All parameters in the GBI calculation were simulated with PHoeBuS software, except for the current
 264 values that were measured experimentally. Note that the generated currents could also be simulated
 265 with PhoeBus, with little deviation from the measured values. In this case, the GBI can be fully
 266 stimulate-able. To ensure calculation accuracy, different corrections were applied for IS calculation,
 267 using ... [55].

268 A clear tendency was observed regardless of the pH tested, where BGEs with low GBI values ($< 2 \times$
 269 10^9) produced medium and high μ_{EO} ($> 20 \times 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$) whereas those with high GBI ($> 5 \times$
 270 10^9) exhibited efficient EOF suppression ($\mu_{EO} < 10 \times 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$). In the GBI range over 2×10^9

271 (see the insert in the Fig. 2), a good correlation between GBI and μ_{EO} ($r^2 = 0.78$) was achieved,
272 demonstrating the relevance of the proposed model. In case of EOF manipulation for electrokinetic
273 preconcentration or separation of target analytes, users can rely on these GBI (or the tendency shown
274 in Fig. 2) to check the relevant buffers for the expected EOF ranges.

275

276 **3.1.2. Large volume sample stacking using EOF modulation**

277 We propose here a new way of LVSS, using modulation of μ_{EO} via BGE's IS and concentrations.
278 The principle behind this EOF-assisted enrichment method is illustrated in Fig. 3. As the EOF is
279 manipulated through IS changes rather than pH, our method allows to select the best pH conditions
280 for the analytes during preconcentration and separation without recourse to any capillary coating. In
281 this case, the low EOF for optimal separation can be generated by playing with different BGE's IS
282 (step 3 in Fig. 3). The use of extremely high BGE concentrations to produce such elevated IS, while
283 not trivial with inorganic ions prone to high current generation, is now feasible thanks to the very low
284 electrophoretic movement of the large weakly charged molecules constituting the BGE. To
285 demonstrate the preconcentration performance of our EOF-assisted method, we showed in Fig. 4 a
286 comparison between our LVSS (sample filling 100 % of the capillary volume) using IS adjustment
287 vs. normal CE (sample filling 7 % capillary volume) of FITC 10 nM. By using a BGE composed of
288 90 mM DEA /CAPS, an EO mobility of approx. $20 \cdot 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ was obtained for CZE of FITC,
289 whereas a much higher EOF (μ_{EO} of $50 \cdot 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$) was necessary during the forefront LVSS
290 step. By comparing the peak height ratios, a 24-fold improvement was achieved. The peak height
291 was used rather than the peak area, as the former reflects better the signal improvement with
292 preconcentration approaches. Indeed, an increase in peak area may be due to peak broadening and
293 velocity decrease rather than stacking effect. The difference in signal improvement factor (24) and

294 injected volume one (14) between LVSS-CE-LEDIF and normal CE-LEDIF of MNPs is due to a
295 certain band broadening observed in CE-LEDIF as the injected volume was quite high (7% of the
296 capillary volume) compared to common ones employed for normal CZE. Some peak broadening and
297 less peak symmetry were observed with LVSS-CE-LEDIF of MNPs. The peak shape degradation,
298 which comes solely from LVSS due to manual polarity switching required to transit the analytes from
299 the preconcentration stage to the separation one, is nevertheless acceptable considering a stacking of
300 the whole capillary volume. Good calibration linearity ($R^2 = 0.9982$ for 5 points from 1 to 20 nM)
301 and excellent reproducibilities for peak areas ($RSD \%_{(n=3)} = 1.75 \%$) and migration time ($RSD \%_{(n=3)} = 0.25 \%$)
302 prove satisfied performance of our proposed enrichment method.

303

304 **3.2. LVSS and CE-LEDIF of CSMNPs**

305 We developed then a new approach based on these preliminary investigations for sensitive detection
306 of CSMNPs, using our coating-free preconcentration strategy coupled with CE-LEDIF. To render
307 fluorescent the carboxylic CSMNPs, a FITC-based fluorophore (fluorescein isothiocyanate-derived
308 3-aminopropyltriethoxysilane) was encapsulated in their silica shell during the synthesis process
309 without changing their size and surface charge [7, 9]. First, the CE-LEDIF conditions were optimized
310 to separate CSMNPs from the residual fluorophore, using BGE composed of either TEA / MOPS
311 (pH 7.4), DEA / MOPS (pH 7.4), TRIS / MOPS (pH 7.4), TRIS / CHES (pH 8.4) or DEA / CAPS
312 (pH 9.7) (Fig. S3 in the ESI). As the CSMNPs were found to be stable in buffers having IS of 100
313 mM [9, 44, 45], this IS was kept constant for all tested BGEs. Indeed, CSMNPs were found stable
314 (with little changes in their sizes) for at least 3 days after dispersion in every tested buffer (see table
315 S2 in the ESI). With TEA/MOPS and DEA/MOPS buffers (traces A, B in Fig. S3), peaks of MNPs
316 could not be observed due to too long analysis time. The Tris/MOPS buffer (trace C) gave a much

317 broadened peak of MNPs. In the cases of Tris/CHES (trace D) and DEA/CAPS (trace E), the
318 separations between MNPs and residual fluorophore were satisfactory. The peak height was however
319 much smaller when using the DEA/CAPS buffer. The best separation and peak shapes were therefore
320 achieved with the TRIS / CHES BGE at IS of 100 mM. The CE profile of CSMNPs (polydispersity
321 index PDI of 0.25) was then compared with that of the commercial fluorescent latex NPs (average
322 size of 34 nm, PDI of 0.129). Two different peak zones were observed (Fig. S4 in ESI). These two
323 NP types display clear differences in their CE profiles (*i.e.* migration time, peak sharpness) which
324 come from their charge-to-size ratios and PDI differences. As the two types of NPs possess similar
325 sizes (*i.e.* average diameter of 30 nm), the difference in their migration times may be more dependent
326 on the variation of their surface charge. The LVSS was then included as a forefront for CE-LEDIF
327 of CSMNPs. As the inclusion of LVSS preconcentration induced peak sharpness degradation due to
328 manual polarity switching required to transit the analytes from the preconcentration stage to the
329 separation one, a further optimization of the LVSS conditions was needed to ensure a good separation
330 between MNPs and the residual fluorophore. Optimization of the LVSS process was then carried out
331 by varying the IS of TRIS / CHES BGE (from 80 to 140 mM, see table S3 in the ESI for the measured
332 zeta potentials of MNPs dispersed in these BGEs) at the inlet and outlet ends of the capillary (Fig.
333 S5). The zeta potential of MNPs can vary depending to the IS and compositions of the dispersion
334 buffers. By increasing the IS from 80 to 120 mM using Tris/CHES buffer (pH 8.4), the zeta potential
335 of MNPs increased from -25.7 mV to -12.9 mV. Accordingly, this led to shifting of their
336 electrophoretic mobilities. The variation of MNPs migration time during optimization of the buffer's
337 IS and pH indeed comes from both changes in EOF magnitudes and MNPs' electrophoretic
338 mobilities. Note again, that effort to optimize the CE and LVSS conditions was carried out at IS
339 ranges not far away from 100 mM. Much higher IS (up to 250 mM), while expected to further

340 suppress EOF, would be detrimental to MNPs stability and provoke possible aggregation of
341 nanoparticles. The best performance was achieved with CSMNPs prepared in TRIS / CHES (IS 10
342 mM, pH 8.4) for preconcentration, and separation under BGE of TRIS / CHES (IS 100 mM, pH 8.4).
343 As can be seen from Fig. S6, the superposition of electropherograms from different runs demonstrated
344 clearly the very good repeatability for LVSS-CZE of CSMNPs under the optimized conditions, with
345 RSD values for migration times of 0.14 % and 2.15 % for the intraday and interday tests, respectively.
346

347 Comparison between CE-LEDIF of CSMNPs with and without LVSS (*i.e.* sample filling 100 % vs
348 7 % of the capillary volume, respectively) was then made (Fig. 5). The signal registered for CE-
349 LEDIF of nanoparticles without preconcentration is very small and close to the detection limit level
350 (Fig. 5B) whereas a high peak was achieved with the inclusion of LVSS. Based on peak height ratios,
351 an improvement of detection sensitivity by 20 folds was estimated with the EOF-assisted
352 preconcentration. The CSMNPs peak form with LVSS- CE-LEDIF was a bit more broadened
353 compared to that for normal CE-LEDIF. The heterogenous population of nanoparticles renders the
354 preconcentration of CSMNPs more challenging than that of a single soluble molecule, as it is more
355 difficult to stack all nanoparticles of slightly different electrokinetic mobilities into a very narrow
356 band. Their peak shapes and resolutions nevertheless are satisfactory for further characterization
357 study. Compared to previously reported methods for CSMNPs detection [40, 43], our CE-LEDIF
358 method without LVSS allows an improvement of 3.5 times (compared to CE-MS) and 17 times
359 (compared to CE-UV) respectively for the detection of the CSMNPs' magnetic cores. With the
360 inclusion of the LVSS preconcentration step, a detection limit improvement of 340 was achieved
361 compared to conventional CE-UV for this purpose.

362

363 Monitoring interaction between nanoparticles and target drugs is an important step for nanomedicine
364 development and drug delivery [56, 57]. Working with a low quantity of nanoparticles for such study
365 is preferable to avoid problems of cytotoxicity and aggregation. Towards this purpose, the potential
366 of the LVSS- CE-LEDIF method to monitor the interaction between CSMNPs at an extremely low
367 concentration and two antibiotics (kanamycin and amikacin) was tested. In this proof-of-concept
368 study, diluted CSMNPs were mixed with different antibiotic concentrations and analyzed by LVSS-
369 CE-LEDIF (Fig. 6). As shown in Fig. 6A, the affinity of the positively charged kanamycin to the
370 carboxylic surface of CSMNPs could be confirmed by the clear shift of migration time and change
371 of peak shape in the presence of kanamycin. On the other hand, the peak shape and migration time
372 remained almost unchanged with amikacin, even at a higher concentration (50 μM) (Fig. 6B). These
373 results point out a probable affinity difference of CSMNPs towards these two antibiotics, with much
374 less interaction between amikacin and the target nanoparticles compared to the case of kanamycin.
375 Note that the antibiotics (*i.e.* kanamycin 20 μM or amikacin 50 μM) were included in both samples
376 and buffers in order to maintain the constant presence of antibiotics at all steps. For the first time, the
377 association equilibrium between nanoparticles and target molecules could be maintained during both
378 electrokinetic preconcentration and separation steps, rather than only during the separation process
379 as in other CE works for such purpose.

380

381 **4. Conclusions**

382 We developed a successful combination of electrokinetic preconcentration via EOF modulation under
383 alkaline conditions and CE-LEDIF separation of nanoparticles. For the first time, EOF can be
384 suppressed down to $2 \cdot 10^{-5}$ ($\text{cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$) in fused-silica capillary without recourse to any capillary
385 coating or gel addition, and can be modulated in a wide range just by changing the BGE's IS. This

386 approach opens the door for different modes of EOF-assisted preconcentration in fused silica
387 capillaries, which have been made possible till now mainly through the use of neutrally coated
388 capillaries. One of its types (i.e. LVSS with polarity switching) was successfully applied to separate
389 and detect CSMNPs, with an improvement of 340 times for detection limit compared to the
390 conventional CE-UV approach. Development of other modes of EOF-assisted preconcentration as
391 well as their exploitations for sensitive and selective determination of biomolecules and nanometric
392 entities with CE-LEDIF are envisaged.

393

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397 some preliminary experiments on EOF suppression.

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411 **Table 1.** Inorganic-species-free BGE compositions at different pH, simulated with Phoebus

412 program

BGE compositions at 150mM	pH	I (μ A) at E = 400 V/cm*	Mobility ($\text{cm}^2/\text{V.s}$)		Buffer capacity (mmol/l.pH)	Remark
			Cation	Anion		
Triethanolamine 150.6 mM + MES 638.6 mM	5.5	28	0.00001	-0.00007	281	To be tested**
Diethanolamine 150 mM + MES 638.6 mM		58	0.00031		279.7	To be tested
Ethanolamine 150 mM + MES 638.6 mM		71	0.00044		279.6	To be tested
Tris 150.3 mM + MES 638.6 mM		57	0.00030		280.3	To be tested
Triethylamine 150mM +MES 638.7mM		63	0.00036		279.6	
Diethylamine 150mM + MES 638.7mM		70	0.00044		279.6	
Trimethylamine 150mM+ MES 638.7mM		76	0.00049		279.6	
Triethanolamine 181.6 mM + MOPS 194.6 mM	7.2	27	0.00001	-0.00021	139.3	To be tested
Diethanolamine 152.4 mM + MOPS 194.6 mM		57	0.00030		85.2	To be tested
Ethanolamine 150.6 mM + MOPS 194.6 mM		70	0.00044		81.1	To be tested
Tris 165.2 mM + MOPS 194.6 mM		56	0.00027		111.4	To be tested
Triethylamine 150mM +MOPS 194.6mM		62	0.00036		79.9	
Diethylamine 150mM + MOPS 194.6mM		69	0.00044		79.8	
Trimethylamine 150.3mM+ MOPS 194.6mM		75	0.00049		80.4	
Triethanolamine 966,5 mM + CHES 872,7 mM	8.7	18	0.00001	-0.00004	488.9	To be tested
Diethanolamine 225,7 mM + CHES 872,7 mM		51	0.00020		414.1	To be tested
Ethanolamine 168,2 mM + CHES 872,7 mM		64	0.00040		340	To be tested
Tris 582 mM + CHES 817,6 Mm		50	0.00007		551.7	To be tested
Triethylamine 151.1mM +CHES 872.7mM		56	0.00036		306.7	
Diethylamine 150.7mM + CHES 872.7mM		64	0.00043		305.8	
Trimethylamine 150.3+ CHES 872.7mM		69	0.00046		323.2	
Diethanolamine 751.2 mM + CAPS 872.5 mM	9.6	36	0.00007	-0.00001	563.7	To be tested
Ethanolamine 294.9 mM + CAPS 872.5 mM		50	0.00024		464.7	To be tested
Triethylamine 158.69mM +CAPS 872.5mM		43	0.00034		322.4	
Diethylamine 155.3mM + CAPS 872.5mM		52	0.00042		315.7	
Trimethylamine 222.28+ CAPS 872.5mM		58	0.00034		410.8	

413

414 * *Electric current simulated for a capillary of 50 μ m ID*415 ** *This simulated BGE was then experimentally tested for EOF suppression with CE-LEDIF.*416 *See equation (1) (GBI calculation) for the logic behind the selection of these BGEs to be*
417 *experimentally tested.*

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423 **Figure captions:**

424 Fig. 1. The measured EOF magnitudes at different pH and IS values. A) pH 9.5-10; B) pH 8.4-
425 8.7; C) pH 7.2-7.4; D) pH 5.5-6.0 Fused silica capillary with ID of 50 μm , L_{eff} of 10 cm,
426 L_{tot} of 40 cm. Sample DMSO 0.1%. Hydrodynamic injection at 50 mbar over 10 s. CE
427 voltage 17kV. See ‘Experimental section’ and table 1 for preparation of the tested BGEs.
428

429 Fig. 2. Correlation between calculated good-buffer-index (GBI) values and the experimentally
430 measured μ_{EO} values for different tested BGE compositions. See ‘Experimental section’
431 and table 1 for preparation of the tested BGEs.
432

433 Fig. 3. Principle of LVSS- CE-LEDIF with our new pH-free EOF manipulation approach.
434

435 Fig. 4. A) LVSS- CE-LEDIF of 10 nM FITC; B) Normal CE-LEDIF of 10 nM FITC (prepared
436 in deionized water). See section ‘Methods’ for LVSS and CE-LEDIF protocols.
437

438 Fig. 5. A) LVSS- CE-LEDIF of CSMNPs (diluted 4000x in Tris/CHES 10 mM); B) CE-LEDIF
439 of CSMNPs (diluted 4000x in Tris/CHES 10 mM). See section ‘Methods’ for LVSS and
440 CE-LEDIF protocols.
441

442 Fig. 6. Electropherograms for LVSS and CE-LEDIF of CSMNPs (diluted 4000x in Tris/CHES
443 10 mM) with / without Kanamycin 20 μM (A) and with / without Amikacin 50 μM (B).

444 Hydrodynamic injection at 50 mbar over 840s to fill 100 % of the capillary volume.
445 Preconcentration was carried out under -25kV (negative polarity) over 5 min. The
446 polarity of the high voltage was then reversed to trigger CZE separation of the stacked
447 CSMNPs in the presence / absence of the tested antibiotics. BGE composed of Tris /
448 CHES (100 mM, pH 8.4) with / without the antibiotics, respectively. Fluorescent
449 detection at excitation and emission wavelengths of 480 nm and 520 nm respectively.

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