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

Ngoc Van Thanh Nguyen¹, Claire Smadja^{1*}, Myriam Taverna^{1,2}, Sirine El Mousli³, Emilie Secret³, Jean-Michel Siaugue³, Lac Thuy Huu Nguyen⁴ and Thanh Duc Mai ^{1*}

¹ *Université Paris-Saclay, CNRS, Institut Galien Paris-Saclay, 92296, Châtenay-Malabry, France*

² *Institut Universitaire de France (IUF)*

³ *Sorbonne Université, CNRS, Physico-chimie des Électrolytes et Nanosystèmes Interfaciaux, PHENIX, F-75005 Paris, France*

⁴ *Faculty of Pharmacy - University of Medicine and Pharmacy at Ho Chi Minh City - 41 Dinh Tien Hoang St., Ben Nghe Ward, District 1, Ho Chi Minh City, Vietnam*

Correspondence: E-mail: thanh-duc.mai@u-psud.fr;

claire.smadja@u-psud.fr

Keywords: capillary electrophoresis; fluorescence detection; stacking; preconcentration; EOF suppression and modulation; magnetic nanoparticles

Abstract

It is reported in this study a new approach for modulation and even suppression of the electroosmotic flow (EOF) to achieve better electrokinetic preconcentration in capillary electrophoresis. This is based on the augmentation of the buffer's concentrations to very high levels (more than a thousand of mM) without recourse to any dynamic/permanent coating nor viscous gel. The use of large weakly charged molecules as background electrolyte's constituents allows working at extreme concentration ranges without penalty of high electric currents and Joule heating. By this way, the electroosmotic 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), and suppressed to levels equivalent to those obtained with several neutral coatings. For demonstration, this new approach was applied for sensitive determination of core-shell magnetic nanoparticles (CSMNPs) having high potential for healthcare applications such as imaging agents for diagnostics and controllable cargos for nanomedicine. Different profiles were achieved for purpose-made and commercial magnetic nanoparticles using CE coupled with light-emitting-diode induced fluorescence (LEDIF) detection. Compared to the conventional capillary electrophoresis (CE-UV) method for characterization of magnetic nanoparticles, our proposed approach with fluorescent detection and EOF-assisted preconcentration offers almost 350-fold sensitivity improvement. Furthermore, our scheme can be used for monitoring the interaction between CSMNPs and target pharmaceutical molecules, serving for drug delivery development. A preliminary study with two antibiotics using this approach revealed that kanamycin interacts better with the target nanoparticles than amikacin.

1) Introduction

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

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

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

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

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

2. Experimental

2.1. Chemicals and reagents

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

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

2.2. Apparatus and Material

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

2.3. Methods

Synthesis and physio-chemical characterization of core-shell MNPs

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

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

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

Measurement of electroosmotic flow (EOF)

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

CE-LEDIF of FITC and core-shell MNPs

These experiments were carried out with the Agilent system coupled with LIF and UV detection. Prior to the first use, the silica capillary (I.D. of 50 μ m, L_{eff} of 50 cm for UV detection, L_{eff} of 35 cm for LIF detection and L_{tot} of 60 cm) was flushed with following sequence: NaOH 1 M for 5 min, DI water for 5 min, then BGE for 25 min. Before each analysis, the capillary was flushed with SDS for 5 min, NaOH for 5 min, then DI water for 3 min and BGE for 5 min. FITC or CSMNPs samples were hydrodynamically injected at 50 mbar for 30 s. FITC samples for CE-LEDIF were prepared by dilution of the stock solution FITC (1000 nM) with deionized water whereas CSMNPs samples were 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 Tris/ CHES (IS 10 mM, pH 8.5). The samples were prepared daily. The separation was performed under 25 kV (normal polarity) at 25 °C. The signal of EOF was recorded by UV detection whereas FITC and MNPs signals were observed by LIF detection. The fluorescent signals (analog signals) were recorded with a data acquisition system and converted into digital ones, which are displayed in the mV scale.

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

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

3. Results and Discussion

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

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

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

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

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

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

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

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

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

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

A clear tendency was observed regardless of the pH tested, where BGEs with low GBI values ($< 2 \times 10^9$) 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 ($> 5 \times 10^9$) 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

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

3.1.2. Large volume sample stacking using EOF modulation

We propose here a new way of LVSS, using modulation of μ_{EO} via BGE's IS and concentrations. The principle behind this EOF-assisted enrichment method is illustrated in Fig. 3. As the EOF is manipulated through IS changes rather than pH, our method allows to select the best pH conditions for the analytes during preconcentration and separation without recourse to any capillary coating. In this case, the low EOF for optimal separation can be generated by playing with different BGE's IS (step 3 in Fig. 3). The use of extremely high BGE concentrations to produce such elevated IS, while not trivial with inorganic ions prone to high current generation, is now feasible thanks to the very low electrophoretic movement of the large weakly charged molecules constituting the BGE. To demonstrate the preconcentration performance of our EOF-assisted method, we showed in Fig. 4 a comparison between our LVSS (sample filling 100 % of the capillary volume) using IS adjustment vs. normal CE (sample filling 7 % capillary volume) of FITC 10 nM. By using a BGE composed of 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, 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 step. By comparing the peak height ratios, a 24-fold improvement was achieved. The peak height was used rather than the peak area, as the former reflects better the signal improvement with preconcentration approaches. Indeed, an increase in peak area may be due to peak broadening and velocity decrease rather than stacking effect. The difference in signal improvement factor (24) and

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

3.2. LVSS and CE-LEDIF of CSMNPs

We developed then a new approach based on these preliminary investigations for sensitive detection of CSMNPs, using our coating-free preconcentration strategy coupled with CE-LEDIF. To render fluorescent the carboxylic CSMNPs, a FITC-based fluorophore (fluorescein isothiocyanate-derived 3-aminopropyltriethoxysilane) was encapsulated in their silica shell during the synthesis process without changing their size and surface charge [7, 9]. First, the CE-LEDIF conditions were optimized to separate CSMNPs from the residual fluorophore, using BGE composed of either TEA / MOPS (pH 7.4), DEA / MOPS (pH 7.4), TRIS / MOPS (pH 7.4), TRIS / CHES (pH 8.4) or DEA / CAPS (pH 9.7) (Fig. S3 in the ESI). As the CSMNPs were found to be stable in buffers having IS of 100 mM [9, 44, 45], this IS was kept constant for all tested BGEs. Indeed, CSMNPs were found stable (with little changes in their sizes) for at least 3 days after dispersion in every tested buffer (see table S2 in the ESI). With TEA/MOPS and DEA/MOPS buffers (traces A, B in Fig. S3), peaks of MNPs 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

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

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

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

4. Conclusions

We developed a successful combination of electrokinetic preconcentration via EOF modulation under alkaline conditions and CE-LEDIF separation of nanoparticles. For the first time, EOF can be 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 coating or gel addition, and can be modulated in a wide range just by changing the BGE's IS. This

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

Acknowledgement

This work has been financially supported by the ‘bourse d’excellence de l’ambassade de France’ (for PhD scholarship of N.V.T. Nguyen). We thank Mr. Ayoub Boulghobra (Université Paris-Saclay) for some preliminary experiments on EOF suppression.

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

* Electric current simulated for a capillary of 50 μm ID

** This simulated BGE was then experimentally tested for EOF suppression with CE-LEDIF.

See equation (1) (GBI calculation) for the logic behind the selection of these BGEs to be experimentally tested.

Figure captions:

Fig. 1. The measured EOF magnitudes at different pH and IS values. A) pH 9.5-10; B) pH 8.4-8.7; 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, L_{tot} of 40 cm. Sample DMSO 0.1%. Hydrodynamic injection at 50 mbar over 10 s. CE voltage 17kV. See ‘Experimental section’ and table 1 for preparation of the tested BGEs.

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

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

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

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

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

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

References:

- [1] M.N. Alves, M. Miro, M.C. Breadmore, M. Macka, Trends in analytical separations of magnetic (nano)particles, *TrAC Trend Anal. Chem.*, 114 (2019) 89-97.
- [2] T.K. Mudalige, H. Qu, D. Van Haute, S.M. Ansar, S.W. Linder, Capillary electrophoresis and asymmetric flow field-flow fractionation for size-based separation of engineered metallic nanoparticles: A critical comparative review, *TrAC Trend Anal. Chem.*, 106 (2018) 202-212.
- [3] L. Trapiella-Alfonso, G. Ramírez-García, F. d'Orlyé, A. Varenne, Electromigration separation methodologies for the characterization of nanoparticles and the evaluation of their behaviour in biological systems, *TrAC Trend Anal. Chem.*, 84 (2016) 121-130.
- [4] S.S. Aleksenko, M. Matczuk, A.R. Timerbaev, Characterization of interactions of metal-containing nanoparticles with biomolecules by CE: An update (2012-2016), *Electrophoresis*, 38 (2017) 1661-1668.
- [5] S. Dziomba, K. Ciura, M. Dawid, The on-line preconcentration of nanoparticles in electromigration techniques, *J. Chromatogr. A*, 1606 (2019).
- [6] H. Schoneborn, F. Raudzus, E. Secret, N. Otten, A. Michel, J. Fresnais, C. Menager, J.M. Siaugue, H. Zaehres, I.D. Dietzel, R. Heumann, Novel Tools towards Magnetic Guidance of Neurite Growth: (I) Guidance of Magnetic Nanoparticles into Neurite Extensions of Induced Human Neurons and In Vitro Functionalization with RAS Regulating Proteins, *J. Funct. Biomater.*, 10 (2019).
- [7] T.D. Mai, F. d'Orlye, C. Menager, A. Varenne, J.M. Siaugue, Red blood cells decorated with functionalized core-shell magnetic nanoparticles: elucidation of the adsorption mechanism, *Chem. Comm.*, 49 (2013) 5393-5395.

- [8] M. Laurencin, N. Cam, T. Georgelin, O. Clement, G. Autret, J.M. Siaugue, C. Menager, Human Erythrocytes Covered with Magnetic Core Shell Nanoparticles for Multimodal Imaging, *Adv. Healthc. Mater.*, 2 (2013) 1209-1212.
- [9] T. Georgelin, S. Bombard, J.M. Siaugue, V. Cabuil, Nanoparticle-Mediated Delivery of Bleomycin, *Angew. Chem. Int.*, 49 (2010) 8897-8901.
- [10] T. Georgelin, V. Maurice, B. Malezieux, J.M. Siaugue, V. Cabuil, Design of multifunctionalized gamma-Fe₂O₃@SiO₂ core-shell nanoparticles for enzymes immobilization, *J. Nanoparticle Res.*, 12 (2010) 675-680.
- [11] B. Teste, F. Malloggi, J.M. Siaugue, A. Varenne, F. Kanoufi, S. Descroix, Microchip integrating magnetic nanoparticles for allergy diagnosis, *Lab Chip*, 11 (2011) 4207-4213.
- [12] D. Baron, P. Dolanska, Z. Medrikova, R. Zboril, J. Petr, Online stacking of carboxylated magnetite core-shell nanoparticles in capillary electrophoresis, *J. Sep. Sci.*, 40 (2017) 2482-2487.
- [13] D. Baron, C. Cacho, J. Petr, Electrokinetic preconcentration of magnetite core - carboxylic shell nanoparticles by capillary electrophoresis, *J. Chromatogr. A*, 1499 (2017) 217-221.
- [14] C. Cacho, Z. Markova, J. Sevcik, R. Zboril, J. Petr, Study of behavior of carboxylic magnetite core shell nanoparticles on a pH boundary, *J. Chromatogr. A*, 1364 (2014) 59-63.
- [15] R.L. Chien, D.S. Burgi, Sample stacking of an extremely large injection volume in high-performance capillary electrophoresis, *Anal. Chem.*, 64 (1992) 1046-1050.
- [16] J.F. Flores-Aguilar, L.C. Medrano, E. Perez-Escalante, J.A. Rodriguez, R.L. Camacho-Mendoza, I.S. Ibarra, Large-volume sample stacking with polarity switching for analysis of azo dyes in water samples by capillary electrophoresis, *Int. J. Environ. Anal. Chem.*, 99 (2019) 1255-1267.

- [17] M. Pieckowski, P. Kowalski, T. Baczek, Combination of large volume sample stacking with polarity switching and cyclodextrin electrokinetic chromatography (LVSS-PS-CDEKC) for the determination of selected preservatives in pharmaceuticals, *Talanta*, 211 (2020).
- [18] Y.X. Shao, G.H. Chen, R. Fang, L. Zhang, L.X. Yi, H.L. Meng, Analysis of Six beta-Lactam Residues in Milk and Egg by Micellar Electrokinetic Chromatography with Large-Volume Sample Stacking and Polarity Switching, *J. Agric. Food Chem.*, 64 (2016) 3456-3461.
- [19] S.Y. Lee, C.E. Muller, Large-volume sample stacking with polarity switching for monitoring of nucleotide pyrophosphatase/phosphodiesterase 1 (NPP1) reactions by capillary electrophoresis, *Electrophoresis*, 35 (2014) 855-863.
- [20] L.X. Yi, G.H. Chen, R. Fang, L. Zhang, Y.X. Shao, P. Chen, X.X. Tao, On-line preconcentration and determination of six sulfonylurea herbicides in cereals by MEKC with large-volume sample stacking and polarity switching, *Electrophoresis*, 34 (2013) 1304-1311.
- [21] A.V. Herrera-Herrera, L.M. Ravelo-Perez, J. Hernandez-Borges, M.M. Afonso, J.A. Palenzuela, M.A. Rodriguez-Delgado, Oxidized multi-walled carbon nanotubes for the dispersive solid-phase extraction of quinolone antibiotics from water samples using capillary electrophoresis and large volume sample stacking with polarity switching, *J. Chromatogr. A*, 1218 (2011) 5352-5361.
- [22] J. Honegr, J. Safra, M. Polasek, M. Pospisilova, Large-Volume Sample Stacking with Polarity Switching in CE for Determination of Natural Polyphenols in Plant Extracts, *Chromatographia*, 72 (2010) 885-891.
- [23] L. Yu, S.F.Y. Li, Large-volume sample stacking with polarity switching for the analysis of bacteria by capillary electrophoresis with laser-induced fluorescence detection, *J. Chromatogr. A*, 1161 (2007) 308-313.

- [24] C.C. Wang, S.S. Chiou, S.M. Wu, Determination of mercaptopurine and its four metabolites by large-volume sample stacking with polarity switching in capillary electrophoresis, *Electrophoresis*, 26 (2005) 2637-2642.
- [25] L. Sola, Chapter 3 - Column Technology for Capillary Electromigration Methods, in: C.F. Poole (Ed.) *Capillary Electromigration Separation Methods*, Elsevier 2018, pp. 45-68.
- [26] L. Hajba, A. Guttman, Recent advances in column coatings for capillary electrophoresis of proteins, *Trac-Trend Anal. Chem.*, 90 (2017) 38-44.
- [27] L.A. Kartsova, D.V. Makeeva, V.A. Davankov, Nano-sized polymer and polymer-coated particles in electrokinetic separations, *TrAC Trend Anal. Chem.*, 120 (2019) 115656.
- [28] D. Corradini, L. Sprecacener, Dependence of the electroosmotic flow in bare fused-silica capillaries from pH, ionic strength and composition of electrolyte solutions tailored for protein capillary zone electrophoresis, *Chromatographia*, 58 (2003) 587-596.
- [29] P.C. Hiemenz, *Principles of Colloid and Surface Chemistry*, Marcel Dekker, New York 1986.
- [30] F. Oukacine, M. Taverna, Suppression of apparent fluid flow in capillary isotachopheresis without recourse to capillary coating, *Analytical chemistry*, 86 (2014) 3317-3322.
- [31] A. Wuethrich, P.R. Haddad, J.P. Quirino, Zero net-flow in capillary electrophoresis using acrylamide based hydrogel, *Analyst*, 139 (2014) 3722-3726.
- [32] J.M. Busnel, A. Varenne, S. Descroix, G. Peltre, Y. Gohon, P. Gareil, Evaluation of capillary isoelectric focusing in glycerol-water media with a view to hydrophobic protein applications, *Electrophoresis*, 26 (2005) 3369-3379.
- [33] T. Tsuda, K. Nomura, G. Nakagawa, Open-tubular microcapillary liquid chromatography with electro-osmosis flow using a UV detector, *J. Chromatogr. A*, 248 (1982) 241-247.

557 [34] B.B. VanOrman, G.G. Liversidge, G.L. McIntire, T.M. Olefirowicz, A.G. Ewing, Effects of
558 buffer composition on electroosmotic flow in capillary electrophoresis, *J. Microcolumn*
559 *Separations*, 2 (1990) 176-180.

560 [35] W.L. Ding, M.J. Thornton, J.S. Fritz, Capillary electrophoresis of anions at high salt
561 concentrations, *Electrophoresis*, 19 (1998) 2133-2139.

562 [36] J.C. Reijenga, T. Verheggen, J. Martens, F.M. Everaerts, Buffer capacity, ionic strength and
563 heat dissipation in capillary electrophoresis, *J. Chromatogr. A*, 744 (1996) 147-153.

564 [37] D.J. Pietrzyk, S. Chen, B. Chanthawat, Enhanced capillary zone electrophoretic separation of
565 dinitrophenyl-amino acid derivatives through control of electroosmotic flow by the buffer
566 cation, *J. Chromatogr. A*, 775 (1997) 327-338.

567 [38] M.M. Bushey, J.W. Jorgenson, CAPILLARY ELECTROPHORESIS OF PROTEINS IN
568 BUFFERS CONTAINING HIGH-CONCENTRATIONS OF ZWITTERIONIC SALTS, *J.*
569 *Chromatogr.*, 480 (1989) 301-310.

570 [39] T.V. Popa, C.T. Mant, R.S. Hodges, Ion-interaction CZE: The presence of high concentrations
571 of ion-pairing reagents demonstrates the complex mechanisms involved in peptide separations,
572 *Electrophoresis*, 28 (2007) 2181-2190.

573 [40] D. Baron, J. Rozsypal, A. Michel, E. Secret, J.M. Siaugue, T. Pluhacek, J. Petr, Study of
574 interactions between carboxylated core shell magnetic nanoparticles and polymyxin B by
575 capillary electrophoresis with inductively coupled plasma mass spectrometry, *J. Chromatogr.*
576 *A*, 1609 (2020).

577 [41] M. Girardot, F. d'Orlye, A. Varenne, Electrokinetic characterization of superparamagnetic
578 nanoparticle-aptamer conjugates: design of new highly specific probes for miniaturized
579 molecular diagnostics, *Anal. Bioanal. Chem.*, 406 (2014) 1089-1098.

580 [42] F. d'Orlye, A. Varenne, P. Gareil, Size-based characterization of nanometric cationic
581 maghemite particles using capillary zone electrophoresis, *Electrophoresis*, 29 (2008) 3768-
582 3778.

583 [43] F. d'Orlye, A. Varenne, T. Georgelin, J.M. Siaugue, B. Teste, S. Descroix, P. Gareil, Charge-
584 based characterization of nanometric cationic bifunctional maghemite/silica core/shell particles
585 by capillary zone electrophoresis, *Electrophoresis*, 30 (2009) 2572-2582.

586 [44] M. Laurencin, T. Georgelin, B. Malezieux, J.M. Siaugue, C. Menager, Interactions Between
587 Giant Unilamellar Vesicles and Charged Core-Shell Magnetic Nanoparticles, *Langmuir*, 26
588 (2010) 16025-16030.

589 [45] R. Massart, Preparation of aqueous magnetic liquids in alkaline and acidic media, *Ieee Trans.*
590 *Magn.*, 17 (1981) 1247-1248.

591 [46] N. Fauconnier, A. Bee, J. Roger, J.N. Pons, Adsorption of gluconic and citric acids on
592 maghemite particles in aqueous medium, in: C. Solans, M.R. Infante, M.J. GarciaCelma (Eds.)
593 *Prog. Colloid Polym. Sci.* 1996, pp. 212-216.

594 [47] B.A. Williams, C. Vigh, Fast, accurate mobility determination method for capillary
595 electrophoresis, *Anal. Chem.*, 68 (1996) 1174-1180.

596 [48] K. Mesbah, T.D. Mai, T.G. Jensen, L. Sola, M. Chiari, J.P. Kutter, M. Taverna, A neutral
597 polyacrylate copolymer coating for surface modification of thiol-ene microchannels for
598 improved performance of protein separation by microchip electrophoresis, *Microchim. Acta*,
599 183 (2016) 2111-2121.

600 [49] T.D. Mai, F. d'Orlye, A. Varenne, A Comprehensive Study of Silanization and Co-
601 Condensation for Straightforward Single-Step Covalent Neutral Capillary Coating,
602 *Chromatographia*, 78 (2015) 775-783.

- [50] C.C. de Lassichere, T.D. Mai, M. Otto, M. Taverna, Online Preconcentration in Capillaries by Multiple Large-Volume Sample Stacking: An Alternative to Immunoassays for Quantification of Amyloid Beta Peptides Biomarkers in Cerebrospinal Fluid, *Anal. Chem.*, 90 (2018) 2555-2563.
- [51] M.U. Musheev, Y. Filiptsev, S.N. Krylov, Noncooled Capillary Inlet: A Source of Systematic Errors in Capillary-Electrophoresis-Based Affinity Analyses, *Anal. Chem.*, 82 (2010) 8637-8641.
- [52] V. Sladkov, Effect of non-thermostated capillary inlet in affinity capillary electrophoresis: Uranyl-selenate system at variable temperatures, *J. Chromatogr. A*, 1263 (2012) 189-193.
- [53] M. Morani, M. Taverna, T.D. Mai, A fresh look into background electrolyte selection for capillary electrophoresis-laser induced fluorescence of peptides and proteins, *Electrophoresis*, 40 (2019) 2618-2624.
- [54] D. Corradini, G. Cannarsa, E. Fabbri, C. Corradini, Effects of alkylamines on electroosmotic flow and protein migration behaviour in capillary electrophoresis *J. Chromatogr. A*, 709 (1995) 127-134.
- [55] *Analys*, PhoEBuS software, http://5.189.177.170/~analys/site/objects/media/0/0/5/0/9/0050933_media/media1.pdf, (1996).
- [56] Y. Wu, Z. Lu, Y. Li, J. Yang, X. Zhang, Surface Modification of Iron Oxide-Based Magnetic Nanoparticles for Cerebral Theranostics: Application and Prospection, *Nanomaterials*, 10 (2020) 1441.
- [57] K. Li, H. Nejadnik, H.E. Daldrup-Link, Next-generation superparamagnetic iron oxide nanoparticles for cancer theranostics, *Drug Discov. Today*, 22 (2017) 1421-1429.