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A B S T R A C T

This study reports on the conception of magneto-Capillary Electrophoresis (magneto-CE), an approach integrating immuno-capture on circulating bio-functionalized magnetic beads into a unique capillary for preconcentration and electrokinetic separation. This hybrid mode is an evolution of in-capillary magnetic bead-based operation from static cluster format to dynamic configuration where beads are allowed to controllably circulate inside a CE capillary for interaction improvement. To implement the magneto-CE operation, a purpose-made instrument was constructed, allowing visual observation of the movement of the magnetic beads. We applied a new methodological strategy for determination of the amyloid β peptide (Aβ 1−42), which is as an established biomarker for molecular diagnosis of Alzheimer's disease (AD). The methodology is based on magneto-immuno-capture of fluorescently labeled Aβ 1−42 followed by a chemical elution with a basic solution prior to CE separation with laser induced fluorescent (LIF) detection. The superiority of this dynamic configuration of magneto-CE was demonstrated for this target analyte, with sample pretreatment and separation being performed in-capillary without any delay in between and without any waste of pretreated sample, which otherwise would not be the case with offline/batch-wise operation.

1. Introduction

One major drawback of capillary electrophoresis (CE) is related to the unsatisfied limits of detection, requiring efficient analyte

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enrichment strategies prior to CE separation. Various preconcentration strategies based on either electrokinetic stacking or focalization through electric field variations, analyte velocity changes, or via solid phase extraction and liquid-liquid systems can be found in some recent reviews [1–3]. To match well the working volumes in the nanolitre scales of CE and to avoid sample loss between pretreatment and separation modules, efforts have been devoted to automated and in-capillary preconcentration techniques [1]. Communications in this direction have been frequently made to electrokinetic preconcentration (refer to Refs. [4–6] for recent applications) and in-line solid phase extraction on monolithic or microparticle supports (see Refs. [7–9] for example). The in-line electrokinetic approaches, although quite efficient, are however generally limited by the capillary volume and by their strong dependency on the sample matrix composition. On the other hand, analyte preconcentration on solid supports is much less affected by such factors and represents a strategy very often exploited to preconcentrate molecules from a biological matrix [10]. In particular, magneto-immunocapture, in which analytes (antigens) are selectively captured by antibodies grafted on magnetic particles offers much higher throughput and ease of manipulation through external magnetic fields [11,12]. The combination of magneto-immunocapture and CE has attracted particular attention as it combines the high enrichment capability of functionalized magnetic beads and the high separation power of CE. So far in-line magnetic bead-based operations (magneto-immunocapture in particular) and CE have been performed by trapping of magnetic particles inside a capillary via permanent magnets [13–18]. Despite interesting performances, two limitations remain for such configurations: i) the trapping of the magnetic beads in a dense cluster hinders the efficient capture of the analytes onto the biofunctionalized surface of the beads which is not directly exposed to the solution, and ii) non-automatic manipulations of the magnets by the operator affecting the reproducibility of the protocol.

In a related context, lab-on-a-chip setups that include magneto-immunocapture/assays have well evolved from statically self-assembled format (*i.e.* magnetic beads immobilized as a cluster in a micro-channel via permanent magnets) [19,20] to bead-circulating configurations allowing significant improvement of analyte capture performance [21–26]. For such purpose, efforts to replace permanent magnets with electronically controllable magnetic tweezers for magnetic beads manipulation have been also reported [25,26]. None of these promising tweezer-based manipulations of beads have nevertheless been implemented in combination with CE. Inspired from microfluidic handling of magnetic beads, it is reported herein the design and development of a novel approach as well as the associated instrumentation (hereafter called magneto-CE) allowing both dynamic magnetic bead-based sample treatment and resolute analytes electrokinetic separation within the same microbore capillary. The advantageous features of the magneto-CE over other non-magnetic in-line enrichment techniques prior to CE separation include no limitation of sample volume for enrichment (compared to electrokinetic preconcentration), ease of introduction and renewal of the magnetic beads into the capillary for in-line sample preconcentration (compared to in-line solid phase extraction on monolithic or microparticle supports). In addition, this novel approach offers high potential for automation, miniaturization and low-cost instrumentation as all operations can be realized within a fused silica capillary using electronically controllable components.

We report also in this study a new methodological strategy, towards subsequent adaptation to magneto-CE, for the analysis of the amyloid β peptide (Aβ 1–42), a validated cerebrospinal fluid (CSF) biomarker for molecular diagnosis of Alzheimer's disease (AD) [27]. Aβ 1–42 in cerebrospinal fluid CSF is normally measured in routine clinical practice using immunoassays [27]; however significant inter-laboratory discrepancies and high relative standard deviation (often over 20%) are frequently observed with the commercial ELISA kits. Mass spectrometry (MS) is an attractive alternative that has been explored with a high detection performance [28–30]. Nevertheless, off-line immune-precipitation is frequently required upstream for enrichment of target peptides and removal of sample matrix [28]. Intense sample pre-treatment(s) and heavy instrumentation are often needed to ensure good MS performances. Quite recently, immuno-enrichment of Aβ 1–42 on magnetic beads followed by electrokinetic separation of the enriched peptide has been considered an interesting and more straightforward approach for AD diagnosis purpose [20,21,31]. The present work is our continued effort towards this objective, using a single capillary for both sample treatment and analyte separation without any volume mismatch nor sample loss. In our original approach, a magneto-immuno-capture of fluorescently labeled Aβ 1–42 is performed, followed by the 'antibody free' determination of this analyte with CE separation coupled to laser induced fluorescent (LIF) detection. To improve the CE-LIF performance, as well as to subsequently adapt to magneto-CE operation, the employment of background electrolytes (BGEs) totally composed of organic ions instead of conventional ones containing low UV absorbing inorganic ions is herein presented. Magneto-CE was then for the first time demonstrated, using the developed methodology, as an alternative to immunoassays for Aβ1-42 measurement with no bias-induced immuno-detection thanks to electrokinetic separation of fluorescently labeled Aβ 1–42.

2. Experimental

2.1. Chemicals and reagents

All chemicals for preparation of buffers were of analytical or reagent grade and purchased from VWR (Fontenay-sous Bois, France). Amyloid beta peptide Aβ 1–42 was purchased from Eurogentec (Seraing, Belgium). The Fluoprobe 488 NHS ester was obtained from Interchim (Montluçon, France). Tris(hydroxymethyl) aminomethane (Tris), 2-(cyclohexylamino)-ethanesulfonic acid (CHES), N-(2-hydroxy-1,1-bis(hydroxymethyl)-ethyl)glycine (Tricine), boric acid, sodium hydroxide, disodium hydrogen phosphate and sodium dihydrogen phosphate were used for preparation of background electrolytes (BGE) solutions.

2.2. Apparatus and material

Method development was performed with a Beckman Coulter PA 800 system (Sciex Separation, Brea, CA) equipped with a solid-state laser induced fluorescence detector ($\lambda_{excitation}$: 488 nm, $\lambda_{emission}$: 520 nm) from Integrated Optics (Acal BFi, Evry, France). Data acquisition and instrument control were carried out using Karat 8.0 software (Sciex Separation, Brea, CA). Polyimide coated fused silica capillaries of 75 μm id and 375 μm od from Polymicro (TSP075375, CM Scientific, Silsden, UK) were used for all separations on the PA 800/LIF instrument. Deionized water was purified using a Direct-Q3 UV purification system (Millipore, Milford, MA, USA). Conductivity and pH values of buffer solutions and samples were acquired with a SevenCompact pH meter (Mettler Toledo, Schwerzenbach, Switzerland). Selection of BGE and buffer ionic strength (IS) calculations were based on simulations with the computer program PhoeBus (Analis, Suarlée, Belgium).

For magneto-CE instrumentation, the sequential injection analysis (SIA), microfluidic manifolds and magnetic tweezers were mounted vertically onto a poly(methyl methacrylate) (PMMA) breadboard with the dimensions of 1 cm (d) x 45 cm (l) x 25 cm (h).

The SIA manifold is based on a syringe pump (Cavro XLP 6000) fitted with a 1 mL syringe and a 9-port channel selection valve (Cavro Smart Valve) purchased from Tecan (Lyon, France). The 3-port valves were purchased from NResearch (HP225T031, Gümligen, Switzerland). The micro-graduated splitting valve was obtained from Upchurch Scientific (P-470, Oak Harbor, WA, USA). All fluid connections were made with 0.02 in. inner diameter (id) and 1/16 in. outer diameter (od) Teflon PFA tubing (Upchurch). The magnetic tweezers were produced in-house according to the design reported elsewhere [25,26]. They are composed of a couple of paramagnetic tips activated by magnetic coils (product No 357−788, RS Components SAS, Beauvais, France). Optocouplers (AQZ102, 60 V 4.0 A) were purchased from RS Components (Beauvais, France). Two cameras for beads observation were purchased from Dino Lite (product no. AM4113ZT, Ludres, France). The electrophoresis module was based on a dual polarity high voltage power supply (Spellman CZE2000, Pulborough, UK) with ±30 kV maximum output. UV transparent coated fused silica capillaries of 75 μm id and 375 μm od from (TSH075375, CM Scientific, Silsden, UK) were used for all operations on the magneto-CE instrument. Detection for the magneto-CE instrument was carried out with a LED induced fluorescence (Zetalif-LED) detector purchased from Picometrics (Toulouse, France). The resulting signal was recorded with a Mini-corder ER180R data acquisition system (eDAQ Europe, Zarszawa, Poland) connected to the USB-port of a personal computer. The programming package LabVIEW (version 8.0 for Windows XP, from National Instruments, Austin, TX, USA) was used to write the control code. The image-processing package ImageJ (https://imagej.net/) was used to calculate the bead cluster areas in the captured images.

2.3. Methods

2.3.1. Dissolution and storage of peptides

Aβ 1−42 was dissolved at 2 mg mL^{-1} in pure DMSO in order to prevent *in vitro* aggregation of the peptide [32]. Aliquot solutions of 5 μL were stored immediately after reconstitution at - 20 °C.

2.3.2. Fluorescent labelling of peptides and sample filtration

The Fluoprobe 488 NHS ester was dissolved in DMSO to obtain aliquots of 10 mg mL^{-1} which were then stored at −20 °C in the darkness. Each aliquot of amyloid peptide Aβ 1−42 was diluted in a sodium borate buffer (pH 10.5, IS 40 mM) containing Fluoprobe 488 NHS to obtain the desired concentration with a molar ratio of 200:1 (Fluoprobe/peptide). After 5 min of incubation at room temperature, successive filtrations on 10 kDa (Amicon Ultra-15 Centrifugal Filter Unit, Millipore (UK) Limited, Hertfordshire, UK) and 3 kDa (Amicon Ultra-15 Centrifugal Filter Unit, Millipore (UK)) membranes were then carried out to remove the excess of fluorophore (see Ref. [33] for the detailed procedure). The peptide retained on the membrane was then recovered into PBS 1×.

2.3.3. Batchwise immunocapture of labeled Aβ 1−42 peptide

Magnetic micro-particles (Dynabeads MyOne Tosylactivated, 100 mg in 1 mL) were coated with monoclonal anti-Aβ antibodies (6E10 or 12F4) according to the manufacturer protocol. The antibodies-bound magnetic beads were subsequently re-suspended at a concentration of 10 mg mL^{-1} in PBS 1× containing BSA 0.1% for storage at 4 °C. A neodymium magnet (Adem-Mag MSV from Ademtech, Pessac, France) was employed to trap the magnetic beads during removal or addition of a suspension solution.

For magneto-immunocapture in batch, the suspension of antibodies-coated magnetic beads was vortexed for 3 min for homogenization before withdrawal of 25 μL aliquots. The suspension solution was removed from the beads that were trapped by a magnet, and a volume of 50 μL of labeled Aβ 1−42 in PBS 1× was added for incubation on a thermo-stated shaker (Thermomixer C, Eppendorf, Montesson, France) at 37 °C for 4 h. The beads were then washed with PBS 1× twice. Subsequently, 10 μL of an elution solution (NH_4OH 0.16%−4%) was pipetted into the washed beads and the suspension was agitated for 10 min at room temperature. The eluent was then separated from the magnetic beads and subjected to CE-LIF analysis without further dilution.

2.3.4. CE-LIF of Aβ 1-42

Analysis of labeled Aβ 1−42 was done with a BGE composed of Tris/CHES (ionic strength I = 20 mM, pH 8.3) using a fused-silica capillary of 75 μm id (the total length L_t of 60 cm and the effective length L_{eff} of 50 cm) under a separation voltage of 20 kV with the normal polarity (*i.e.* positive polarity at the injection end). Before use each day, the fused silica capillaries were preconditioned with 1 M NaOH for 10 min and deionized water for 10 min prior to flushing with buffer. The capillaries were then used continuously for successive analyses within one day. Deionized water was used for the preparation of all solutions.

3. Results and discussion

3.1. Design of magneto-CE

To overcome the actual limitations encountered with existing immuno-enrichment modules based on functionalized magnetic beads and coupled to CE separation (i.e. working volume mismatch between sample treatment and separation steps and modest immuno-capture performance with static bead clusters), a novel approach called magneto-CE is proposed for the first time. With magneto-CE, the capillary serves directly as a micro-reactor for immuno-enrichment of target analytes using circulating beads, and at the same time for in-line CE separation. The schematic outline of the magneto-CE methodology is demonstrated in Fig. 1. Bio-functionalized magnetic beads are first injected into the capillary via hydrodynamic injection of the bead suspension, and then trapped or released thanks to two pairs of magnetic tweezers positioned close to the two ends of the capillary. By alternatively switching on/off the two magnetic tweezers and passing back and forth a hydrodynamic flow of a certain sample volume through the capillary, the magnetic beads can circulate between the two pairs of magnetic tweezers (steps 1−4) in order to allow analytes immunocapture. After this dynamic immuno-capture step, the magnetic beads retaining target analytes are washed with a BGE flow of a certain volume, in order to remove non-specifically adsorbed molecules on magnetic beads. For the elution step, the beads are then trapped near the grounded end of the capillary, and a plug of the elution solution is hydrodynamically injected into the capillary and delivered to the bead zone for elution (step 5). Then, the released analytes are immediately analyzed with CE in the same capillary under a high voltage without any loss of sample (step 6). The normal polarity (anode at the injection end) was set for the particular case of Aβ 1-42 separation as this species is negatively charged under basic conditions and is dragged by the electro-osmotic flow (EOF) from (+) to (−) towards the detector [21]. Fig. 1 serves also as a general illustration of magneto-CE that may be applied to other species than Aβ 1−42. The polarity may therefore be changed according to the analyte(s) of interest. After the CE separation, beads are removed from the capillary by a capillary flushing with BGE while the magnetic tweezers are deactivated (step 7).

The in-capillary immuno-extraction in a dynamic suspension of bio-functionalized magnetic beads so far cannot be realized on any

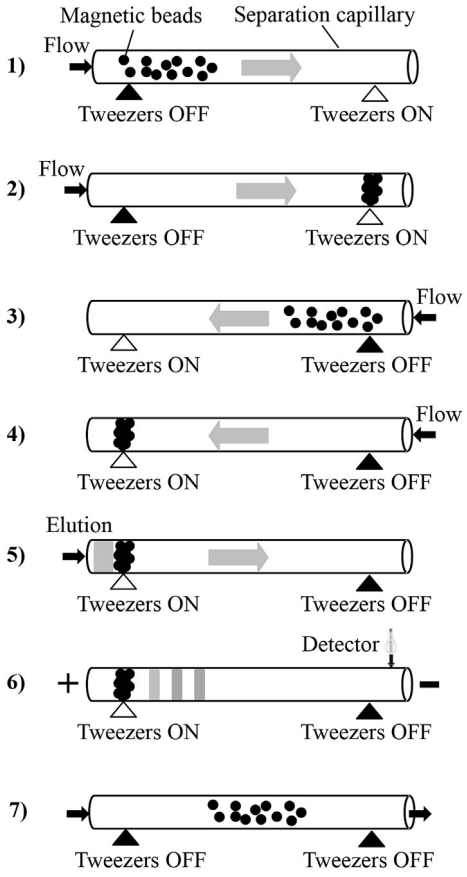

Fig. 1. Concept of in-capillary immuno-enrichment on circulating bio-functionalized magnetic beads for capillary electrophoresis.

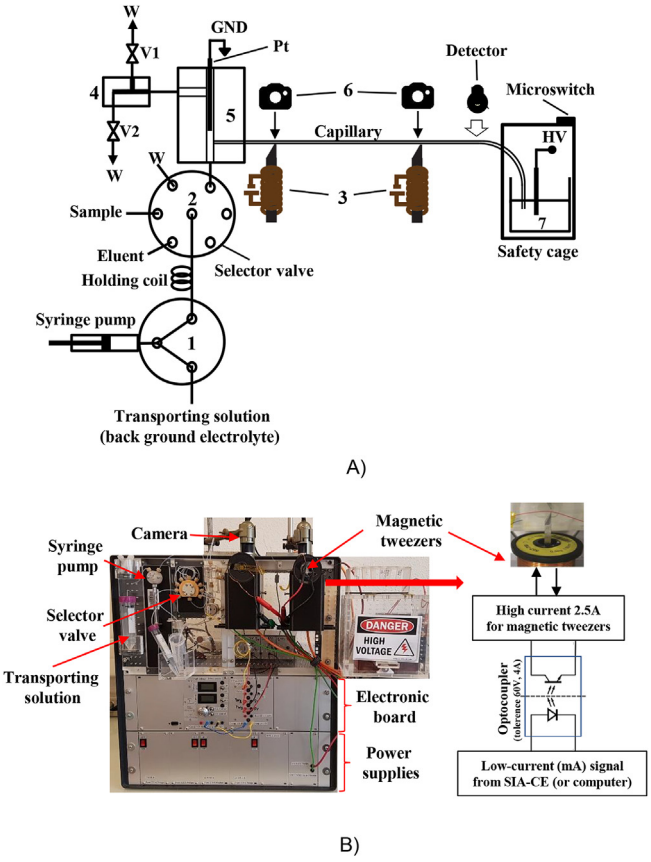

Fig. 2. A) Simplified schematic drawing of the magneto-CE instrument. HV: high voltage; GND: electrical ground; Pt: platinum electrodes; W: waste; V1, V2: electrically actuated isolation valves; BGE: background electrolyte; Pt: Platinum electrode. 1) Stepper motor-driven syringe pump; 2) Multiple-port selection valve; 3) Magnetic tweezers; 4) Graduated splitting valve; 5) Fluidic interface; 6) Cameras; 7) HV vial containing either beads suspension, sample solution or BGE.
B) Photo of the magneto-CE instrument, and the schematic drawing of magnetic tweezers with their control using an electronic optocoupler.

commercial CE instrument. Thus, a purpose-made system that hyphenates microfluidic, electro-magnetic and electrophoretic operations was constructed. With this in-lab-built instrument, automatic control and direct observation of the movement of the magnetic beads inside a CE capillary were made possible. A simplified schematic drawing of the system is shown in Fig. 2A. Extensions and modifications have been made to our previous SIA–CE design [34,35] in order to incorporate the magneto-immunocapture into the system. A combination of a stepper motor-driven 2-way syringe and a multi-port selector valve was used for delivery of solutions. Triggering of the high voltage for electrophoresis, and manipulation of two magnetic tweezers were realized via auxiliary Transistor-Transistor Logic (TTL) pins of such syringe and selector valve. A fluidic interface machined in a perspex block (see details in Ref. [34]) as well as blocking valves were employed for magnetic bead injection, back and forth sample manipulation, hydrodynamic injection of the eluent and flushing of the capillary. The ground electrode was arranged along the fluidic channel and perpendicular to the capillary end inside the fluidic interface (see point 5 of Fig. 2A). Compared to the previous setup [34] where the ground electrode was positioned perpendicularly to the fluidic channel, this new arrangement allows to maximize the contact of the electrode with the electrolyte. With the previous interface setup, we observed current ruptures due to casual formation and accumulation of bubbles inside the fluidic channel. This bubble formation indeed can disrupt the electrical contact between the ground electrode and the capillary during electrophoresis. In the new setup where the ground electrode and the fluidic channel are in a vertical position (Fig. 2A), air bubbles emerged upward and did not interfere the electrical contact between the ground electrode and the capillary situated beneath. A graduated needle valve, acting as a split injector, was employed to adjust the amount of the eluent or sample to be injected into the capillary. To allow a tiny volume of bead suspension to pass through and be trapped inside the capillary, hydrodynamic injection of beads suspension was carried out from the HV end of the capillary. This was done with a backward flow created by the precise pulling action from the syringe pump on the closing of two isolation valves (V1 and V2) to block the outlets of the fluidic interface. For delivery of the magnetic beads and sample solution during the immuno-capture-elution steps, the controlled back and forth movement of the syringe piston was employed to create a backward or forward flow. Two pairs of magnetic tweezers were positioned at two sides of the capillary (5 cm from the ground side, and 20 cm from the HV side) to allow magnetic beads to circulate in the capillary zone defined by these two magnetic tweezers.

To assure a good mechanical stability of the instrument, as well as to facilitate modification and extension during design optimization, the whole system was constructed as independent modules and assembled into a standardized 19 in. rack (see Fig. 2B). The power supplies including the high voltage power for electrophoresis and electronic interfaces were arranged in two rack-mounted withdrawable cases at the bottom. The fluidic components and magnetic tweezers are fixed onto a perspex breadboard (25 cm (h)

x 45 cm (l) x 1 cm (d)) situated above the two electronic rack inserts. Two pairs of magnetic tweezers made from a couple of paramagnetic tips activated by a magnetic coil ([25,26]) were observed with two cameras and white light-emitting-diode (LED) back light illumination. Optocouplers with a current load of 4 A maximum were used to isolate the low power signal (mA) circuitry controlling the syringe pump, valves and electrophoresis parts from the high-power section of the magnetic tweezers (up to 2.5 A) (Fig. 2B).

3.2. Operation and performance

The details of a typical magneto-CE workflow are given in Table 1. In this protocol, beads are aspired into the capillary from the HV end, followed by a back and forth circulation between two magnetic tweezers in a flow of sample. Elution is carried out from the ground end of the capillary prior electrokinetic separation without any delay between these two steps. The capture and release of magnetic beads inside a capillary with magnetic tweezers are shown in Fig. 3A. Magnetic beads are well dispersed in the absence of a magnetic field and circulate along the capillary instead of staying in a packed cluster as observed when permanent magnets were used. Compared to our previous microfluidic setup where beads were allowed to circulate in much larger channels (at least 300 μm) [25,26], beads circulation is expected to be more efficient due to the very narrow diameter (75 μm) of the capillary channel. We showed also that upon activation of magnetic tweezers, magnetic beads were trapped into a very compact cluster (Fig. 3A). To verify the reproducibility of the bead trapping process, magnetic tweezers were used to repeat 5 cycles of magnetic beads' capture and release. Fig. 3B presents the bead recoveries which were calculated from the areas of bead clusters captured at the tweezers as a function of the number of capture/release cycles. A good capture performance was ensured after 3 cycles, where the magnetic force was always superior to the drag force induced from the hydrodynamic flow during the capture process. After 4 or 5 cycles bead recoveries drastically decreased probably due to hydrodynamic drag force becoming superior to the magnetic one. This could be explained by the fact that these magnetic tweezers were initially designed for microfluidic operations with much shorter activation time (<1 min). In our case, the trapping time (via activation of a high electric current) is much longer (several minutes), leading to excessive heating of magnetic tweezers. At the same time, the hydrodynamic flow created by the stepper syringe may not be perfectly smooth all the time. Small hydrodynamic pulses may lead to some partial loss of magnetic beads after each cycle especially when magnetic tweezers are overheated. To solve the overheating problem, miniaturization of the tweezers allowing less power consumption and heat generation could be an option. To avoid the hydrodynamic pulses, combination of the stepper-motor driven syringe pump and a flowrate sensor with a flowrate regulation system could be an interesting alternative (through more sophisticated systems) to maintain a more stable flowrate during in-capillary bead circulation.

3.3. Methodological development for determination of fluorescently labeled Aβ 1–42 peptide

Aβ 1–42 peptide is an established biomarker for molecular diagnosis of Alzheimer's disease, whose concentrations in cerebrospinal fluids (CSF) are at the sub nM ranges. To improve the detection sensitivity (with LIF rather than UV detection), fluorescently labeled Aβ 1–42 is preferably analyzed [21,33,36]. In our previous works on CE/MCE-LIF coupled with magneto-immunocapture, in which at least one step was performed offline, two strategies were already investigated for Aβ 1–42 tracing, featured by (i) capture - labeling - elution by heating at 95 °C [21] and (ii) capture - chemical elution under acidic conditions - labeling [20]. As direct magneto-immuno-capture of fluorescently labeled Aβ 1–42 followed by chemical elution has not previously been implemented, this novel approach was explored in this study. We optimized CE-LIF conditions to achieve at the same time efficient stacking and good separation of Aβ 1–42 from the residual fluorescent dye in the eluent. We also investigated the conditions allowing good immuno-recognition of the modified Aβ 1–42 and its efficient elution without degrading the fluorescent signal due to quenching or hydrolysis of the labeled peptide.

3.3.1. BGE optimization for CE-LIF of labeled Ab 1-42

For CE-LIF analyses, normally BGEs composed of inorganic species (typically phosphate and borate/NaOH buffers), which possess no UV absorbing features, are employed. Due to the quite high conductivities of such BGEs, high concentrations are not recommended to minimize the Joule heating effect. This on the other hand may result in less efficient stacking of analytes from sample matrices having high conductivity/high ionic concentrations, which in turns adversely influence the separation resolution and detection sensitivity. In another context, BGEs composed of very high concentrations of large and weakly charged organic species and in the absence of inorganic counter ions are often used for CE with

Table 1
Typical operation protocol of the magneto-CE system.

Step	Operation	Syringe movement direction	V1	V2	MT 1	MT 2	HV	Injection position
1	Flushing the capillary with BGE	forward	closed	closed	Off	Off	Off	GND side
2	Injection of magnetic beads	backward	closed	closed	On	Off	Off	HV side
3	Rinsing the interface with sample	forward	open	open	On	Off	Off	GND side
	Sample injection and immuno-capture	forward	closed	closed	Off	On	Off	GND side
	Sample injection and immuno-capture (back circulation)	backward	closed	closed	On	Off	Off	HV side
4	Rinsing the interface with BGE	forward	open	open	On	Off	Off	GND side
	Rinsing of trapped magnetic beads and capillary with BGE	forward	closed	closed	On	Off	Off	GND side
5	Rinsing the interface with eluent	forward	open	open	On	Off	Off	GND side
	Injection of an eluent plug	forward	closed	open	On	Off	Off	GND side
6	Rinsing the interface with BGE	forward	open	open	On	Off	Off	GND side
	Delivery of the eluent plug to magnetic beads (for elution)	forward	closed	closed	On	Off	Off	GND side
7	Electrophoretic separation	Off	closed	closed	On	Off	On	–
8	Rinsing the interface with BGE	forward	open	open	On	Off	Off	GND side
	Removal of magnetic beads and flushing of capillary with BGE	forward	closed	closed	Off	Off	Off	GND side

V: valve.
MT: magnetic tweezers.
HV: high voltage.

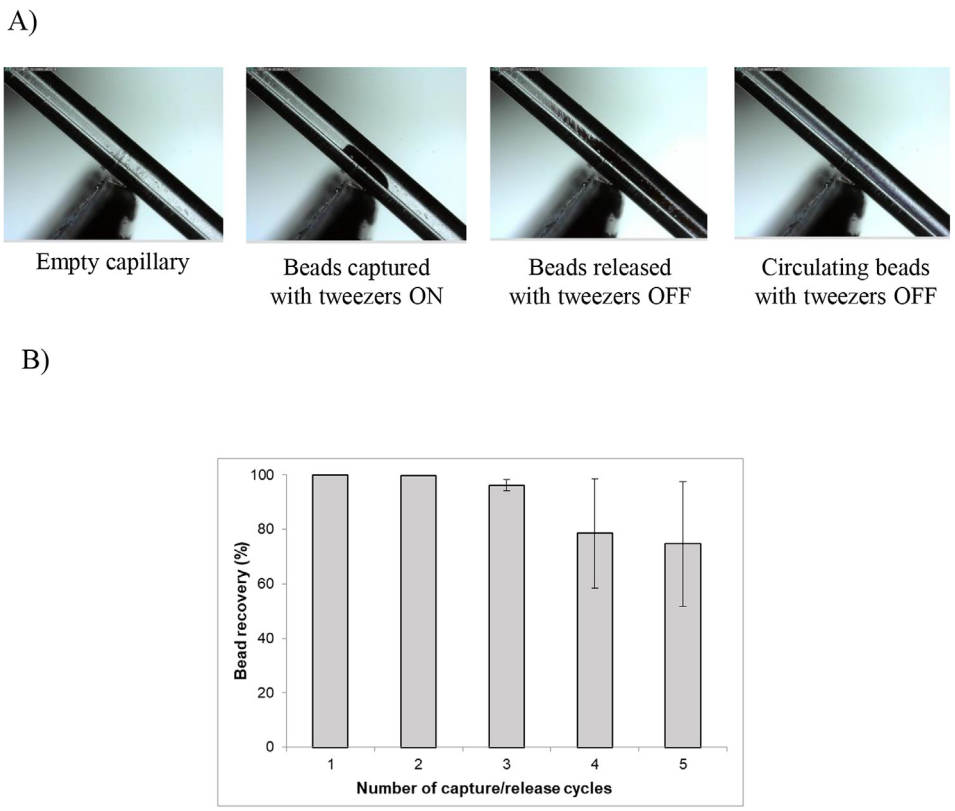

Fig. 3. A) Photos of bead capture and release inside a transparent capillary (75 μm ID and 375 μm OD) with the activation/deactivation of magnetic tweezers; B) Bead capture performance, calculated from the areas of bead clusters captured at the tweezers over 5 consecutive activation/deactivation cycles.

conductivity detection [37,38]. Such BGEs provoke a maximum mismatch of the conductivity and ionic density between the BGE and sample matrix zones for efficient stacking as well as minimize unwanted Joule heating effect thanks to their very low current generation in the capillary. While these BGEs are not favorable for CE with UV detection due to the high background signals induced from their UV absorption, they would offer much improved performance for CE-LIF separation as they do not produce residual detection signals at the working excitation wavelength (normally in the visible range). Fig. 4 shows the separation of mono-tagged and di-tagged Aβ 1–42 forms from the residual fluorescent dye in the eluent using both CE-LIF conventional inorganic BGEs and Tris/CHES based buffer. This test was carried out with a sample matrix containing 1% ammonium hydroxide, which is the eluent used to release labeled Aβ 1–42 from the functionalized magnetic beads. As can be seen, much sharper and higher peaks of labeled Aβ 1–42 were achieved with the BGE composed of 66 mM Tris and 195 mM CHES (pH 8.3) compared to the conventional borate (20 mM NaOH/113 mM boric acid) and phosphate (1.96 mM NaH_2PO_4/6 mM Na_2HPO_4) electrolytes. At the same ionic strength of 20 mM, such organic BGE produced an at least twice lower current (10 μA) than the other inorganic ones. This organic ion - based BGE with the excellent stacking efficiency and minimal current generation was therefore employed for further CE-LIF analyses of Aβ 1–42 after the immuno-capture step.

3.3.2. Batch-wise magneto-immunocapture and chemical elution of labeled Aβ 1-42

For immunoassays of Aβ peptides, the anti-β-amyloid antibody 6E10 that is specific to amino acid residue 1–16 of beta amyloid (N-term) was used. The immunocapture of labeled Aβ 1–42 was nevertheless found not possible with this antibody (data not shown), probably due to an epitope masking by the fluorophore bound to Aβ 1–42 via a chemical reaction at its N-terminus. The immunocapture of this modified Aβ 1–42 was then carried out with the antibody 12F4 that recognizes specifically Aβ 1–42 at C-term (2nd amino acid). Successful capture of this peptide on magnetic beads grafted with 12F4 antibody was achieved in bulk, as shown in Fig. 5. As the elution was not possible under acidic conditions due to the loss of the fluorescent signal, basic solutions were selected as eluents. Different concentrations of ammonium hydroxide (0.16%–4%) were tested as eluents, and the elution fractions were analyzed by CE-LIF using the developed method. It was found that the eluent composed of 1% ammonium hydroxide at pH 11.3 offered the best elution performance in terms of peak height and sharpness (Fig. 5). Thanks to the high concentrations of organic species in the BGE, excellent stacking of Aβ 1–42 was maintained even with quite a high ammonium hydroxide concentration in the eluent. With such conditions, a calibration curve was acquired with satisfactory correlation coefficient ($r^2 > 0.98$) for the Aβ 1–42 concentration range of 2–40 nM. The reproducibility of the inter-batch measurements of peak areas and migration times was found to be about 8% and 0.5%, respectively. They deemed satisfactory considering that these RSD values are due to the accumulation of errors of all operations, i.e. sample preconcentration, elution, injection and separation.

3.4. In-line immunocapture of labeled Aβ 1-42

In-line immunocapture of labeled Aβ 1–42 (10 nM) on magnetic beads grafted with 12F4 antibody and elution of the captured peptide with 1% ammonium hydroxide in a fused silica capillary,

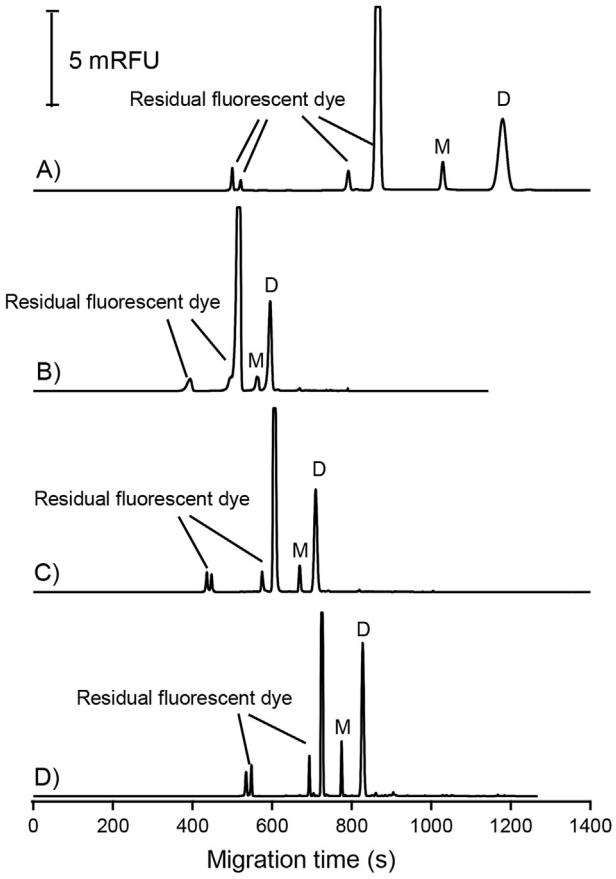

Fig. 4. CE-LIF analysis of labeled Aβ 1–42 (80 nM prepared in 1% ammonium hydroxide) with different BGE compositions. A) Phosphate buffer (I = 20 mM, pH 7.4); B) Borate buffer (I = 20 mM, pH 8.4); C) Tricine/NaOH buffer (I = 20 mM, pH 8.4); D) Tris/CHES buffer (I = 20 mM, pH 8.3). CE conditions: fused silica capillary with l_{eff} = 50 cm, l_{total} = 60 cm, internal diameter of 75 μm; high voltage of 20 kV with normal polarity; LIF excitation at 488 nm and emission at 520 nm. M: mono-tagged Aβ 1–42; D: di-tagged Aβ 1–42; RFU: relative fluorescence unit.

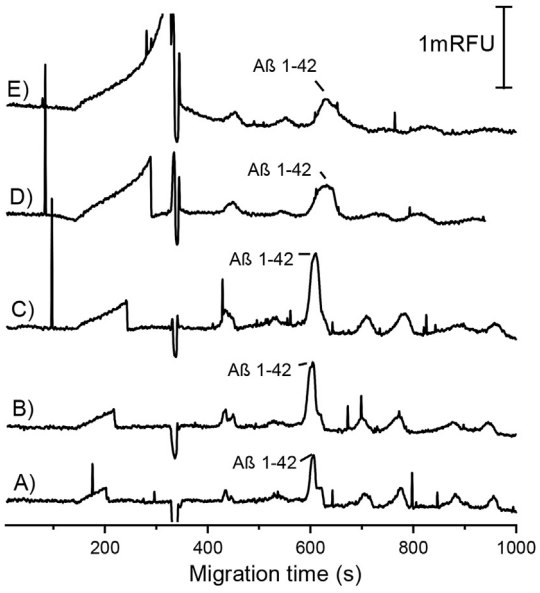

Fig. 5. CE-LIF analysis of labeled Aβ 1–42 that was batch-wise immuno-captured on magnetic beads grafted with 12F4 antibody and eluted with ammonium hydroxide solutions of different concentrations. Elution with A) 0.16% ammonium hydroxide; B) 0.5% ammonium hydroxide; C) 1% ammonium hydroxide; D) 2% ammonium hydroxide or E) 4% ammonium hydroxide. Other CE-LIF conditions as in Fig. 4.

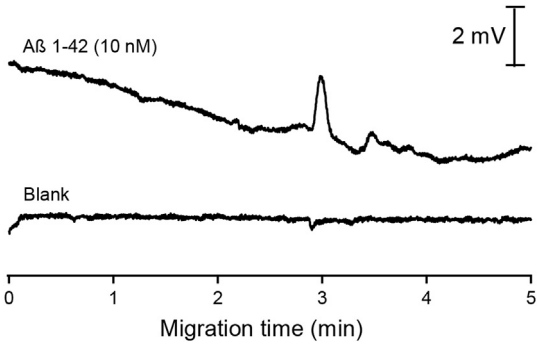

Fig. 6. Determination of labeled Aβ 1–42 (10 nM) with in-line immuno-capture on circulating beads followed by CE-LIF using the magneto-CE instrument. The output signal was automatically converted to electronic unit (mV) when using the Mini-Corder data acquisition system.

followed by CE-LIF analysis of the released Aβ 1–42 was carried out with the developed magneto-CE instrument. The fluorescently labeled Aβ 1–42 used was labeled off-line using a protocol already described which produces mainly the di-tagged form [33,39]. CE-LIF analysis of the eluent was implemented immediately after the elution step in order to limit the fluorescent tag cleavage that may occur under alkaline conditions. Conveniently, the BGE composed of Tris/CHES performed well with the magneto-CE system, thanks to its very low current generation preventing bubble formation due to overheating inside the enclosed fluidic interface. No current rupture was observed with the Tris/CHES buffer when running with the magneto-CE instrument, which was not the case when inorganic BGEs were tested. The CE-LIF profile after in-line magneto-immuno-capture with circulating beads and elution of labeled Aβ 1–42 is shown in Fig. 6. In this case the totality of the eluent is transferred to the CE separation, which is not the case with off-line CE operation where most of the eluent after the immuno-capture step is wasted. On the contrary, no signal was observed when immuno-capture of labeled Aβ 1–42 was carried out with the beads immobilized as a cluster at the tweezers 1 rather than being recirculated in the capillary, demonstrating the added value of using the recirculating beads to increase capture efficiency. Having the beads in a circulating suspension instead of a conventional packed bed is indeed to favor their contact with the analytes (*i.e.* labeled Aβ 1–42) in the solution, thus significantly improving interaction efficiency [22,25,26], while limiting the back pressure generated by the bed of beads. Note that the profiles in Figs. 4 and 5 were obtained with a commercial instrument with an effective separation length of 50 cm whereas those for Fig. 6 were achieved with the purpose-made magneto-CE system having an effective separation length of 35 cm (i.e. the distance between the first magnetic tweezers and the detector). Also, we observed that when working with Aβ 1–42 samples that had passed through magneto-immunocapture and elution prior to CE separation, the peaks were broader compared to those of Aβ 1–42 standards directly injected into a CE system without the sample treatment step. To improve the peak resolution of peptide with the magneto-CE instrument, the separation distance should be increased. Miniaturization of the tweezers thus is envisaged so that a pair of miniaturized tweezers could be positioned closer to the injection end. For the same purpose, the safety cage for high voltage isolation could also be made smaller so that the detector can be positioned closer to the high-voltage end in the next prototype version.

4. Conclusions

The magneto-CE concept and the associated instrument was for the first time developed and successfully demonstrated for in-line magneto-immuno-preconcentration and CE-LIF separation of fluorescently labeled Aβ 1−42 peptide. This approach could render CE-LIF as a performing alternative to conventional immunoassays for detection of protein- and peptide-based biomarkers in biological matrices. At the actual stage of in-house assembled system, the present work represents a first proof-of-concept of the applicability of such a new approach for immunocapture and also its superiority toward immobilized cluster of beads. Miniaturization of magnetic tweezers and adaptation of this miniaturized version to a commercial CE system is under progress to render magneto-CE more robust and widespread.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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