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Development of a Poly(dimethylsiloxane) Interface for On-Line Capillary Column Liquid Chromatography−Capillary Electrophoresis Coupled to Sheathless Electrospray Ionization Time-of-Flight Mass Spectrometry

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An interface in elastomeric poly(dimethylsiloxane) (PDMS) for on-line orthogonal coupling of packed capillary liquid chromatography (LC) (i.d. = 0.2 mm) with capillary electrophoresis (CE) in combination with sheathless electrospray ionization (ESI) time-of-flight mass spectrometric (TOFMS) detection is presented. The new interface has a two-level design, which in combination with a continuous CE electrolyte flow through the interface provides integrity of the LC effluent and the CE separation until an injection is desired. The transparent and flexible PDMS material was found to have a number of advantages when combined with fused silica column technology, including ease to follow the process and ease to exchange columns. By combining conventional microscale systems of LC, CE, and ESI−MS, respectively, the time scales of the individual dimensions were harmonized for optimal peak capacity per unit time. The performance of the LC−CE−TOFMS system was evaluated using peptides as model substances. A S/N of about 330 was achieved for leucine-enkephaline from a 0.5 μL LC injection of 25 μg/mL peptide standard.

On-line analytical systems possess a number of attractive attributes, such as time saving, automation, and a suitable way for sample handling. In this field, systems that incorporate sample preparation in series with separation and detection as well as systems that combine different separation techniques have been developed. Multidimensional separation systems are especially interesting if the analytical processes involved have the prospective of being individually optimized. For complex samples, for example, in proteomics, a single one-dimensional separation step is often incapable of handling the large number of components present in the sample. Combinations of different dimensions of on-line separation may therefore be used to increase the resolving power. In an orthogonal two-dimensional (2D) separation system, the two integrated analytical techniques should have different separation mechanisms[1] as in, for example, 2D gels and the MudPIT[2] approach. Integrating the separation mechanisms of hydophobicity in LC and charge in CE thus provides a truly orthogonal 2D-separation system. In addition, the resolution achieved in the first separation needs to be maintained in the second dimension, a fact that requires accurate sampling between the two dimensions. In 2D gels and MudPIT the total elution volume from the first dimension can be transferred to the second dimension, resulting in a duty cycle of one.[3] In on-line LC−CE, a fast comprehensive sampling ensures that all the components are reanalyzed and that the first separation is adequately characterized. The analysis time of the second dimension is, if not multiplexed, usually considerably faster compared to the first dimension. LC−CE accomplishes these requirements since LC has a high sample loading capacity and operates in the minute time scale, while the complementary CE has a high speed of analysis with a possibility to complete separations within seconds. The enhanced performance of a multidimensional system can be described by dramatically increased resolving power, since the overall peak capacity is the product of the peak capacities in each individual separation step.[1]

Jorgenson and co-workers are responsible for the innovations in the LC−CE field during the 1990s.[4−7] The flow-gating interface was found advantageous compared to the loop-valve system since it eliminates sample collection, storing, and out-flushing between each analysis.[5] More recently, the flow gating interface has been used in other fields of application, including coupling LC on-line to CE immunoassays,[8,9] coupling of microdialysis sampling to CE,[10] and for coupling CE−CE.[11] Robson et al. have also described an interface for LC−capillary electrochromatography (CEC) that, in

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principle, also can be used for LC−CE.[12] The different interfaces have been developed for various detection systems, where only one example involves MS detection.[13] The challenge to adequately sample the narrow peaks from the CE to the MS is, as pointed out by Lewis et al., just as challenging as it is for the CE to sample the LC effluent.[13] The time-of-flight mass spectrometer will however provide fast sampling and does not have the limitation of scanning mass spectrometers, as it registers the whole mass spectrum within a tenth of a second time frame. The sheathless ESI interface is the best choice due to its compatibility with flow rates in the nanoliter per minute range and since it does not introduce any postseparation band broadening effects when the separation device and the emitter are unified. Compared to the earlier reported LC−CE−MS coupling strategy,[13] which used a sheathflow micro ESI needle, the sheathless interface has higher demands on CE electrolyte composition while it provides advantages of being a less complex and a more sensitive system.

The reduced chromatographic dilution gained by using miniaturized LC columns has been well documented,[14] and also in CE, the dimensions are continuously decreasing. CE techniques are today commonly integrated in microfabricated devices, for example, microchips and soft microstructures, providing advantages in miniaturization, reduced reagent consumption, speed, high-performance analysis, and the possibility to produce disposable devices at low costs.[15] Two challenges with the microfluidic devices are the short length of the separation channel that limits the resolving power and how to continuously introduce sample onto the microstructure. A combination of well-established fused silica capillary columns and microfluidic structures might thus be advantageous. Two examples of innovations in this field are a capillary cross-connector, which enables use of CE columns of variable lengths[16] and a connecting capillary for continuous sample introduction onto CE channels in a chip.[17,18] An alternative is to combine the features of elastomeric materials, for example, PDMS with the advantages of hard structures such as fused silica capillaries.[19−21] PDMS thus makes a sufficient tight connection around capillaries, which eliminates the need for nuts, ferrules, and epoxy glue. Compared to the ordinary used switching valve, this type of connection provides low dead volumes, easier deactivation, and higher flexibility and should be useful for coupled LC and CE.

This study describes a simple construction of an LC−CE interface in PDMS. In this work, a previous reported PDMS connection for on-line flow injection analysis (FIA)-CE[19] has been further developed. The effect that the downscaling of the CE inlet construction had on the CE current was evaluated. The innovations were focused on a stabilization of the CE current and the coupling to fast sheathless ESI−TOFMS detection. The performance of the LC−CE−TOFMS system was evaluated, using peptides as model substances.

EXPERIMENTAL SECTION

Reagents. Peptide standards were purchased from Sigma-Aldrich (St. Louis, MO). In positive injection mode, the CE electrolyte consisted of phosphate buffers of pH 2 with an ionic strength of 5 mM or pH 7 with an ionic strength of 50 and 10 mM. In negative injection mode the CE electrolyte consisted of 10 mM acetic acid/acetonitrile (ACN) (50/50, v/v). The LC buffer consisted of 20 mM formic acid/ACN (90/10 mobile phase A, 10/90 mobile phase B, v/v). The CE column deactivation reagent 3-aminopropyl-trimethoxysilane (APS), 97%, was from Sigma Aldrich (Chemie GmbH, Steinheim, Germany). All other chemicals were of p.a. grade or higher and purchased from the usual commercial sources. Solutions were made with deionized water purified with a MilliQ system (Millipore, Bedford, MA).

LC Column Packing Procedure. Fused silica capillaries (l = 100−120 mm, o.d. = 0.36 mm, i.d. = 0.20 mm) were slurry packed with 5 μm Kromasil C_{18} particles, pore size 100 Å (Eka Nobel AB, Bohus, Sweden); 20 mg of packing material was suspended in 0.2 mL of ACN. A pump (PU-980, Jasco, Tokyo, Japan) delivered a flow of ACN through a packing reservoir at a flow rate of 0.1 mL/min until the pressure reached 350 kg/cm^2. This pressure was maintained for 1 h.

CE Column Preparation. Fused silica capillaries (l = 200−250 mm, o.d. = 0.36 mm, i.d. = 0.050 mm) (Polymicro Technologies, Phoenix, AZ) were sharpened at both ends with a grinding paper and a drill. On-column electrodes were produced on the injection ends according to the "Black Dust" method.[22] Bare fused silica capillaries and APS-columns[23] were used for CE. The original APS-column producing procedure was slightly modified. The silanols were activated by 6 M HCl for 2 h. The capillaries were then flushed with toluene and nitrogen for 30 min, respectively. APS (5%) was dissolved in toluene (v/v) and pushed through the capillaries during 4 h. Finally, the capillaries were flushed with toluene and nitrogen for 30 min, respectively. For MS experiments, a thin layer of polypropylene containing graphite was applied to the sharpened detection end to produce an emitter for the electrospray ionization.[24]

LC−CE Interface Preparation. The PDMS structure was cast using fused silica capillaries as templates and a Falcon Petri dish of polystyrene as a holder (35 × 10 mm, Becton Dickinson, Plymouth, UK). Five holes, A−E (0.5 mm), were drilled in the Petri dish. A−D were symmetrical, while E was placed between two holes, see Figure 1. A capillary (o.d. = 0.36 mm) was placed through holes A and B. A small capillary (o.d. = 0.18 mm) was inserted into a larger capillary (o.d. = 0.43 mm, i.d. = 0.32 mm), and this combination was placed through holes C and D, on top of and in right angle to the A−B capillary. These capillaries, in contact with each other, created a two-level template. The distance

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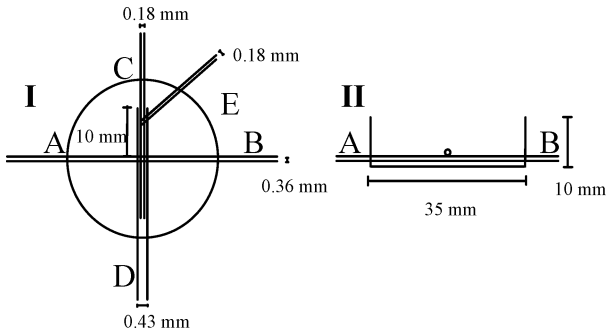

Figure 1. Schematics of the template for casting of the PDMS-interface: (I) top-view and (II) side-view. Dimensions are specified in the figure.

between the tip of the large capillary in the C−D plane and the cross was 10 mm. An additional capillary (o.d. = 0.18 mm) that touch the large capillary was fixed by hole E, between the tip and the cross. Elastosil RT 601 A was mixed with Elastosil RT 601 B (Wacker Kemi AB, Stockholm, Sweden), 10:1, and left for degassing for about 50 min (10 min sonication included) to remove bubbles. The polymeric solution was poured over the capillary template, and possible remaining bubbles were adjusted not to be in contact with the templates. The PDMS was cured in an oven at 60 °C for 30 min before the capillaries were pulled out, leaving a two-level channel structure. All capillaries used as templates were rinsed with methanol on the outside before use to facilitate the removal after casting. A silanization of the internal channels, according to an earlier reported procedure,[25] was performed. First, the channel surfaces were activated with 1 M NaOH for 24 h. APS/water-solution (2.5%) (v/v) was pushed through the channels by a syringe and incubated for 1 h. The channels were rinsed with water, air, and CE electrolyte (10 mM acetic acid/ACN (50/50, v/v)) before use.

Instrumentation. Figure 2 shows a schematic picture of the entire instrumental setup.

Chromatographic System. The separation was performed using a gradient of mobile phase A and B delivered by an Ultra-Plus II LC pump from Micro-Tech Scientific Inc. (Vista, CA) at a flow rate of ∼2 μL/min (split from about 40 μL/min). The gradient was controlled by 2-D Pump Control Software, version 5.0, and went from 100% to 70% of A in 8 min (including 1 min of focusing after injection). Thereafter the column was equilibrated for 15 min. An electrically actuated valve performed the injections, and the injection volume was ∼0.5 μL.

Electrophoresis System. A Bertan ARB 30 (Bertan High Voltage, Hicksville, NY), high-voltage (HV) supply was used. All CE experiments were performed using an on-column electrode,[19] to which the high voltage was applied by a metal string at a distance of ∼20 mm from the tip. A conventional CE setup, where the injection end with the integrated electrode was placed in a buffer vial, was used to produce reference experiments. CE experiments were performed using both positive (positive injection mode) and negative injection potentials (negative injection mode). In positive injection mode bare fused silica capillary CE columns and a potential of +8 kV were used. In negative injection mode, APS-columns were used to produce a reversed EOF at a potential of

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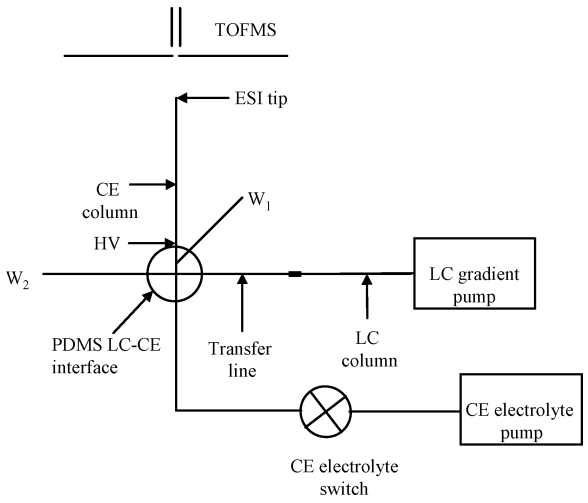

Figure 2. Schematic of the instrumental setup around the PDMS interface for LC−CE. The LC pump delivered a gradient flow of 2 μL/min through the LC column (l = 120 mm, o.d. = 0.36 mm, i.d. = 0.2 mm). The CE column (l = 200−250 mm, o.d. = 0.36 mm, i.d. = 0.050 mm), W_1, and the capillary providing CE electrolyte were positioned in the upper level of the PDMS structure, while the LC transfer line (l = 700 mm, o.d. = 0.36 mm, i.d. = 0.050 mm) and W_2 were plugged into the lower level. The CE electrolyte valve controlled the supply of electrolyte (2 μL/min when switched on and zero if switched off) to the interface. W_1 and W_2 had 0.36 mm o.d. and 0.075 and 0.095 mm i.d., respectively. See Figure 3 for a more detailed illustration of the interface.

−15 kV. The approximated injection volume for hydrodynamic injections, in the conventional CE setup, and for electrokinetic injections, when the CE column was mounted in the interface, was 10−50 nL.

LC−CE Interface. Figure 3 shows an expanded view of the central region of the PDMS interface. For CE electrolyte delivery, a fused silica capillary (o.d. = 0.43 mm, i.d. = 0.32 mm) was inserted in the upper level, position D, and positioned at a distance ∼2 mm from the cross. The LC flow entered the lower level at position B by a fused silica capillary (o.d. = 0.36 mm, i.d. = 0.050 mm) positioned ∼1 mm from the cross. Two waste capillaries (W) (o.d. = 0.36 mm) were used. W_1 (i.d. = 0.075 mm) was plugged in at position E, next to the CE column, and W_2 (i.d. = 0.095 mm) was placed at position A, opposite to the LC flow. A Black Dust coated injection end of the CE column was inserted into the PDMS interface, at position C, opposite to the CE electrolyte flow. This channel is tight in the outer part (0.18 mm in diameter) and broad in the internal part (0.43 mm in diameter). A CE electrolyte flow was delivered by a HPLC pump (PU-980, Jasco, Japan, Tokyo), at a flow rate of 2 μL/min (split from 20 μL/min). An injector (Valco, Schenkon, Switzerland) with a sample loop of 7.8 μL was used to define a liquid sample plug during the interface development. The injection from the LC flow was performed electrokinetically by manually switching off the CE electrolyte flow, during 5 s, using a PEEK switching valve, V-100L (Upchurch Scientific, Oak Harbor, WA). The applied high voltages for the separation and ESI were maintained during injection. A 9-peptide standard (25 μg/mL) was injected into the LC system in LC−CE experiments. The LC column was connected to the LC−CE interface by a fused silica capillary transfer line (l = 700 mm, i.d. = 0.050 mm), as seen in Figure 2, to prevent back currents. Injection from the LC effluent to the CE was performed 2 or 3 times per minute. Repeated

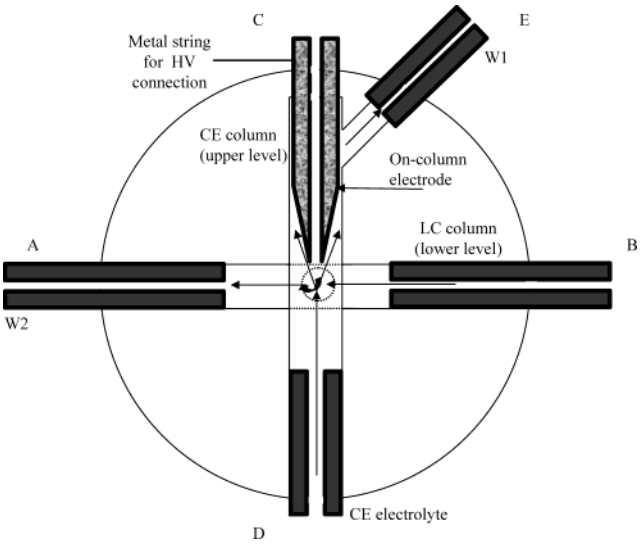

Figure 3. View of the central region of the PDMS flow paths. The Black Dust coated CE column in the upper level was positioned close to the center of the interface, about 200 μm above the LC flow. A metal string provided the contact for the high voltage. A small flow of CE electrolyte swept around the CE-column entrance. The main part of the CE electrolyte left however the interface through W_2. The LC column effluent entered in the lower level and was directed to waste through W_2. Injections were performed electrokinetically by switching off the CE electrolyte during 5 s.

injections were performed during the LC separation window, of about 6−7 min.

Detection. UV detection at 210 nm, using a μ-Peak Monitor (Amersham Biosciences, Uppsala, Sweden) with optical fibers (Polymicro Technologies) (200 μm) and a detector cell built in-house[26] was used for optimization. A Jaguar O-TOFMS instrument (Leco, St. Joseph, MI) equipped with the ChromaTOF software, version 1.0, was used for MS detection. The instrument has a nonreflectron flight tube with quadratic field profile, giving a resolution over 2000 for reserpine (609 m/z). The repulsing frequency was set to 5 kHz and the summing rate to 400, yielding a sampling rate of 12.5 spectra/second. The ESI voltage was optimized for each emitter and varied between 2900 and 3500 V, and the potential on the interface plate was fixed at 500 V. The spray emitter was positioned approximately 5 mm on-axis from the orifice, and the flow of nitrogen curtain gas for the source region was set to 250 mL/min at an orifice temperature of 100 °C.

Safety Considerations. ACN is combustible, volatile, and slightly toxic if exposed to lungs and skin. All acids and bases should be handled with great care. The CE column-coating reagent APS is highly toxic. The peptides used are biologically active and should be handled using protective gloves. To avoid electrical shock, the high voltage power supplies should be handled with extreme care.

RESULTS AND DISCUSSION

Implementation of the LC−CE interface in the analytical system was favored by the well-adapted time scales. The time scale for the LC separation, the CE separation, and the TOFMS is minutes, seconds, and tenth of seconds, respectively. In that way they adequately sample from each other and provide optimal peak capacity per unit time. In this first report on the LC−CE interface, the performance was evaluated by using peptides as model substances. When combining a pressure driven flow with an electrical driven flow, it is important to address special attention to some parameters. The main concerns described in this study involve flows, buffer composition, volumes, material, electrochemistry, injections, and sheathless ESI−TOFMS. The CE current was affected by many of these parameters, and its stability was therefore used as a measure of quality in the optimization. A stable CE current was defined as a constant current during at least 10 min. Conventional CE experiments were used to provide reference values when evaluating the LC−CE interface.

An important issue in an LC−CE connection is that the flow rates, interfacing each other, are independent. While one fraction is being analyzed by the CE system, the next fraction should not enter the CE capillary, or the CE analysis will be obstructed.[27] A constant flow of CE electrolyte, as in the flow-gating interface,[5,7] provided a continuous supply of ions for stabilization of the CE current and prevented electromigration of the LC effluent during a CE run. The two-level structure of the PDMS connection provided additional flow integrity. Compared to earlier reported LC−CE interfaces that used a transverse flow of 300−600 μL/min,[5,7] a CE electrolyte flow rate of 2 μL/min was, in the present interface, sufficient to continuously flush the interface. This was probably due to the small contact area (~0.03 mm^2) of the channels in the two-level interface. The dimensions of the CE column and the waste capillaries were used to regulate the flow dynamics in the LC−CE interface. The chosen dimensions allowed the LC flow to pass right through the connection as long as the CE electrolyte flow was on, see Figure 3. The LC flow and the main part of the CE electrolyte were then passed to waste through W_2. This was to ensure that the LC flow did not continuously leak into the CE capillary. By directing CE electrolyte from the upper level, the contact area between the planes was thoroughly cleaned, and the integrity of the meeting flows was maintained. A minute flow was also directed to pass W_1, which enabled both a CE electrolyte flow to sweep around the CE column and to create a small overpressure in the connection. This overpressure produced a possible hydrodynamical flow in the CE column that was usually restricted to be very low (<1 nL/min) to give minimal effect to the CE separation. This flow was sometimes increased (up to 85 nL/min) to facilitate a positive injection mode. The flow profiles in the interface were controlled and visually confirmed by injecting a dye in the LC stream. As expected, the dye passed right through the connection when the CE electrolyte flow was on. When the CE electrolyte was stopped a small amount of dye was spread up to the injection end of the CE column. This principle was tested as an injection technique, and the primary results, not reported here, were very promising. To further confirm the flow integrity, repeated electrokinetical injections (−10 kV, 5 s) of 100 μg/mL leucine-enkephaline from a liquid plug were performed once every minute. The result presented in Figure 4 shows distinct peaks without any distortion. The EOF in the CE column was found to have a significant effect on the performance, and a high EOF, about 130−200 nL/min, was required to achieve a stable CE

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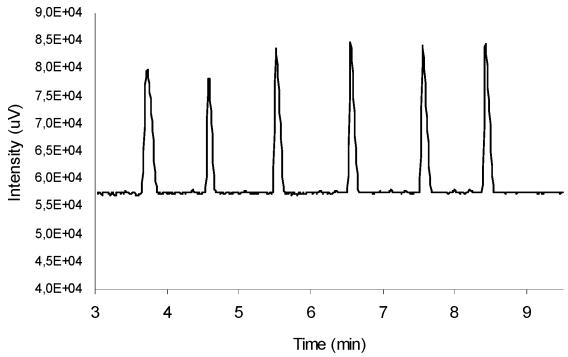

Figure 4. Repeated electrokinetical injections of leucine-enkephaline. The peptides were dissolved in 80% ACN to a concentration of 100 μg/mL. Injections (−10 kV, 5 s) were performed once every minute from a liquid sample plug (7.8 μL) passing at a flow rate of 2 μL/min. About 5 ng was injected each time. APS-coated CE columns and UV detection (210 nm) were used. The first and the last peaks correspond to the front and the end of the sample plug.

current in the connection. In negative mode, APS-coated CE columns provided the necessary high EOF, with high stability over the 10 min test period. For CE separations of peptides using untreated fused silica columns, in positive mode, the commonly used pH 2 phosphate buffer generated, however, an EOF that was too low to produce a stable CE current in this device. EOF was increased by using a phosphate buffer of pH 7 with an ionic strength of 50 mM, which resulted in a current stability that almost reached the 10-min criterion set in this study.

The material used for the interface was PDMS due to the advantages of easy fabrication, transparent properties, and excellent fit and refit for fused silica capillaries. Interfacing fused silica capillaries with microstructures is advantageous since it combines high-resolution CE separations with the additional functionality and flexibility of the microfluidic manifolds. Direct capillary connections also allow easy interfacing with external fluid delivery and detection instruments. Trapped bubbles were easily diagnosed and rectified due to the transparent feature of the PDMS material, and a precise positioning and alignment of the capillaries was easily obtained. Capillaries can readily be plugged in and exchanged in the elastomeric structure, allowing use of separation columns of variable lengths. The PDMS structure also gave tight fittings of largely various o.d. of fused silica capillaries, for example, capillaries could favorably be plugged into channels with 50% smaller diameters. As long as care was taken when exchanging the capillaries, the PDMS interface was intact, and the same device could be used for several weeks. In initial current stability studies and in development of injection techniques, the PDMS surface was used without any pretreatment. For LC−CE analysis of peptides eluting from an LC gradient, APS modification was used to make the surface of the internal PDMS channels less hydrophobic. The APS coating also provided a positively charged surface deactivation that facilitated peptide injection independent of sample matrix. The degree of surface coverage and the degree of optimal surface charge density on the PDMS walls are objectives for future studies. Adsorption to the untreated hydrophobic PDMS surface is quite expected and has been reported elsewhere,[28] and the extent of peptide adsorption and alternative ways of deactivating the PDMS surface is currently also being investigated.

In this study, the integrated electrode, presented in previous work,[19] was used to provide the electrical contacts. The distance between the metal string for HV connection and the CE column tip was shown not to affect the CE performance when varied in the range of 0.5−4.0 cm. For the MS detection, the potential in the detection end of the CE column was adjusted to about +3 kV for the ESI and held at ground potential for UV detection. The electrical field to perform a CE separation was established by applying a positive (+8 kV) or negative (−15 kV) high voltage to the interface electrode. The volume of CE electrolyte in the connection is limited, compared to a conventional CE setup, and this kind of electrode therefore produces a high current density that is important to consider. A high current density can result in the electrolysis of water and thus cause bubble formation. In the small volume of the LC−CE interface, there is a high probability for the bubbles to obstruct the CE separation by covering the inlet of the CE column. The rate of gas bubble formation, for a defined area, is known to be proportional to the current. Low current densities are preferred, since other, non-gas-producing, reactions can be favored. The current density was estimated by dividing the measured CE current with the geometric electrode area and was thus reduced by different strategies to produce a stable CE current. Compared to the first reported FIA−CE interface,[19] the present LC−CE interface provided a 19 times increase in electrode area, which is expected to result in a corresponding decrease in the current density. Assuming a constant current, the current density using a phosphate buffer of pH 7 and ionic strength of 50 mM was calculated to 0.21 and 4.1 mA/cm^2 in the LC−CE and FIA−CE interfaces, respectively. For a defined area, for example, the sharpened tip of the electrode, a 19 times theoretical decrease in the rate of gas bubble formation should be expected. The formation of gas bubbles was however difficult to measure, and the CE current was used as a quality measure to investigate any improvements. In this case, the current was found to be stable for at least 10 min in the LC−CE, compared to about 3 min in the FIA−CE interface. The decrease of the current density was also accomplished by reducing the ionic strength of the CE electrolyte from 50 to 10 mM for a phosphate buffer of pH 7 (positive injection mode). This lowered the current density from 0.21 to 0.037 mA/cm^2 for the LC−CE interface. In negative injection mode, using APS capillaries and MS compatible buffer with even lower ionic strength, 0.2 mM, the current density was reduced to 0.019 mA/cm^2. A stable current throughout the LC−CE runs was thus more easily achieved. Compared to the FIA−CE interface,[19] the improvements in CE current stability in the LC−CE interface were also accomplished by the additional flow around the electrode, that also helped to remove bubbles.

Initially, techniques for injection of analytes into the CE column were developed by making repeated injections of defined fractions from a liquid plug consisting of standard peptides. To make an electrokinetic injection the CE electrolyte flow was switched off, and a small and defined plug from the peptide standard flow, passing through the lower PDMS microchannel, was thus drawn

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into the CE column, see Figures 2 and 3. The high voltages of CE and ESI could remain on during injection without the need for slew up/down periods.[5,7] Consequently, the injection voltage was the same as the separation voltage. This injection technique gave a typical repeatability of about 7% ($n = 4$), calculated on peak area, when injecting 100 μg/mL leucine-enkephaline (−15 kV, 5 s) once every minute from a liquid plug using UV detection. In the LC−CE−TOFMS system, this injection technique gave a S/N value of about 330 for leucine-enkephaline when a peptide standard, at a concentration of 25 μg/mL, was injected in the LC system. The lowest detectable concentration (MDC_{sample}) of leucine-enkephaline, in the overall system, was approximated to 300 ng/mL (or 540 nM). The average response in the chromatoelectropherogram ($n = 2$) was used in estimating the sensitivity and extrapolating to zero. The detection limit was defined as 4 times the peak-to-peak intensity of the noise and measured for a blank injection. In this initial performance test of the interface, the volume injected into the CE from the two-level structure could not be well defined. An alternative injection technique is therefore suggested in future developments including the following series of steps: (a) HV and the CE electrolyte flow switched off, (b) HV switched on for injection (for 5 s) and off again, and (c) CE electrolyte flow and separation HV switched on. With this approach it would be possible to use different injection and separation potentials to provide low-volume injections, but the system will also require automation for optimal performance. In the present study, the manual setup where the high voltages were left on was thus used, providing faster injections and more frequent sampling to the second separation dimension.

An important aspect of LC−CE is the ability to improve the resolution of components present in complex peptide mixtures. A theoretical measure of the performance of multidimensional separations is peak capacity. The peak capacity of a separation with unit resolution is defined as

$$N_c = \frac{L}{4\sigma}$$

where L is the total time (or distance) over which the sample zones (peaks) are distributed and σ is the average standard deviation of the peaks. In the system reported here, the peak width (4σ) for a peptide eluting from the LC was about 0.29 min and the peaks eluted over a range of 3.4 min, which gave a peak capacity of 12. The peaks from the CE had a base width of approximately 5 s and eluted within 22 s, giving a peak capacity of about 4. The chromatographic peak capacity in this system for the analysis time of 3.4 min is therefore 48. Some claim that the MS can be used as an additional dimension,[2,29,30] though it may be questionable to use detector selectivity as a measure of peak capacity. To compare with other multidimensional techniques using MS detection, the peak capacity in the MS was calculated as the number of MS spectra acquired during a chromatographic peak.[2] The TOFMS has an inherent peak capacity of at least 62.5, corresponding to the number of spectra acquired during a CE peak. The total peak capacity in the LC−CE−TOFMS is then 3000 for the 3.4 min LC run, that is, corresponding to 900 min^{-1}. This result may be related to other multidimensional approaches using MS detection, such as, for example, MudPIT[2] where the overall peak capacity for a protein digest was reported to be as high as 23 000 but, due to the long analysis time, corresponding to a value of 17 min^{-1}. To utilize the peak capacity in a 2D system, it is important that the transfer between the integrated techniques match each other in time. The CE was sampled every 0.08 s by the TOFMS, and an injection from the LC effluent was performed every 20th second, as illustrated in Figure 5. With this sampling frequency the LC peak was sampled 1−3 times for subsequent CE separations. As pointed out by Seeley,[3] the sampling period should be less than the $2 \times s_{peak}$, indicating that the sampling frequency should preferably be doubled for an adequate sample of the LC peaks in this system. A higher sampling frequency can be achieved by increasing the speed of the CE separation or by using overlapping injections.[13]

The sheathless ESI process restricts the choice of buffer ions in both the LC and CE separation. The combination of volatile acetic acid/ACN CE electrolyte with APS-coated columns enabled a stable ESI that was optimized for detection of peptides. A separation of a 9-peptide standard containing 25 μg/mL of each peptide, injected in the LC loop, demonstrated the performance of the LC−CE−ESI−TOFMS system. The 2D plot, in Figure 5A, was reconstructed from the combined chromatoelectropherogram by subtracting the CE migration time to estimate the LC retention times. The figure clearly shows that all detected peptides are fully separated in the 2D system, and some peptides are sampled into the CE up to three times. Their relative order in the chromatoelectropherogram follows the LC retention. This verifies that the CE, used as second dimension, is fast enough for taking snapshots. Although eight peptides could successfully be transferred, substance P never reached the detector. It is well-known that the basic substance P is difficult to analyze, especially by PDMS,[31] and possible explanations could either be low efficiency in the LC separation or insufficient deactivation of the PDMS surface. Deactivation of the interface is presently pursued for this purpose. The high quality of information achieved with this multidimensional technique using MS detection is illustrated in Figure 5C, where the m/z spectrum of [arg8]-vasopressin is shown.

CONCLUSIONS

A new interface for LC−CE−MS has been developed. The LC and CE electrolyte flows meet in a two-level structure with a small contact surface. This construction of interface and the CE electrolyte flow assured that the LC flow did not continuously leak into the CE column. The interface is manufactured in elastomeric PDMS that forms tight connections around fused silica capillaries. Advantageous is also the transparent feature of PDMS that facilitates troubleshooting. To achieve a stable current in the interface, a low current density and a high EOF were required. The LC−CE−TOFMS system was evaluated with respect to sensitivity and peak capacity. The lowest peptide concentration that was possible to inject to the system was approximated, for

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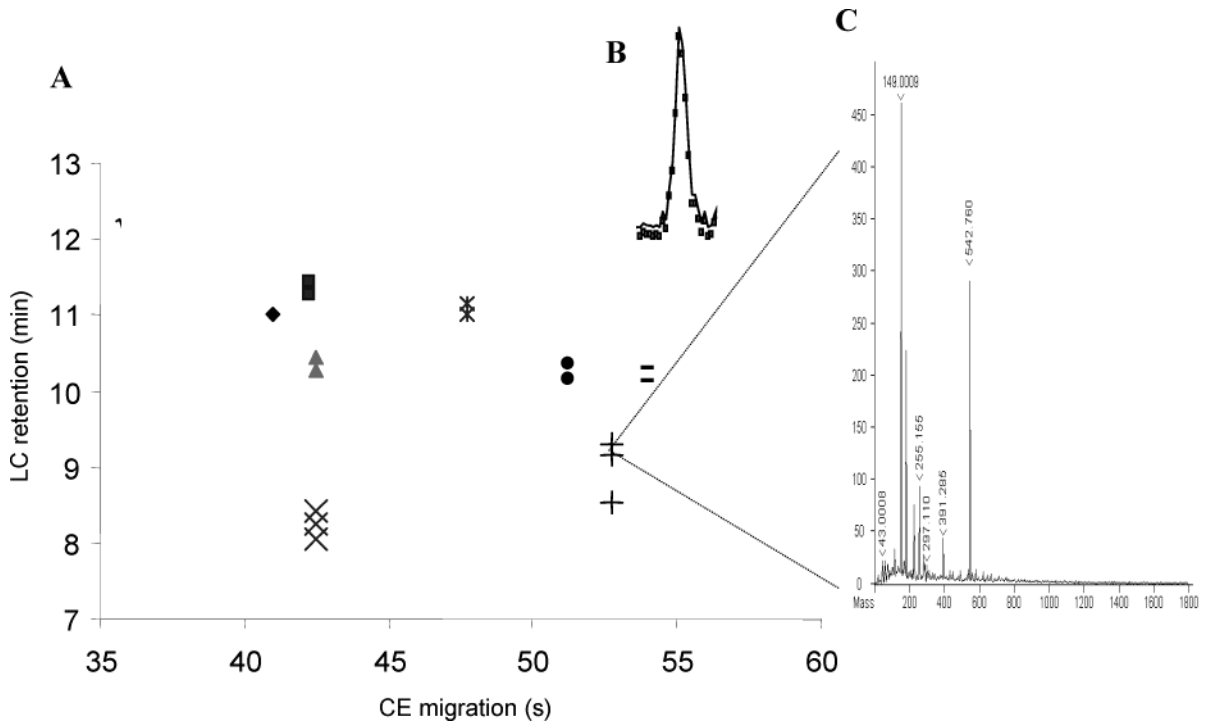

Figure 5. LC−CE separation of a peptide standard. A 9-peptide standard, 25 μg/mL of each peptide, was injected in the LC loop and analyzed by the LC−CE−TOFMS system. Injections from the LC to the CE were performed every 20th second. (A) A 2D representation of LC retention and CE migration of an LC−CE run. The symbols represent the following peptides; ◆ methionine-enkephaline, ■ leucine-enkephaline, ▲ oxcytocin, × bradykinin 1−5, ∗ bombesin, ● luteinizing-hormone-releasing-hormone, + [arg8]-vasopressin, and − bradykinin. (B) A one second broad CE peak sampled by the TOFMS at a speed of 12.5 spectra/second. (C) An *m*/*z*-spectra of [arg8]-vasopressin sampled at an LC time of 9.20 min.

leucine-enkephaline to about 300 ng/mL (corresponding to 540 nM). The chromatographic peak capacity was 48 for an analysis time of 3.4 min, and in combination with mass spectrometric detection the system proved to be very powerful.

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