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Electrosonic spray ionization—An ideal interface for high-flow liquid chromatography applications

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ABSTRACT

Electrosonic spray ionization (ESSI) has been studied as an interface between high-performance liquid chromatography (HPLC) and mass spectrometry (MS), using sample flow rates up to 3.0 ml min^{-1}. This ionization interface was compared with pneumatically assisted electrospray ionization (ESI) using mass spectrometry for detection. For experiments that did not involve direct comparison of different flow rates, the ESI experiments were performed using post column splitting to work at optimal conditions. ESSI allows the interfacing of conventional or high-resolution liquid chromatography (LC) methods to mass spectrometry without post column splitting. High sample flow rates could be handled without a significant loss of signal intensity using a nebulization gas flow rate of 5.5 L min^{-1}. Since ESI needs to be operated with lower sample flow rates, it is limited to micro/nano LC systems, or post column splitting must be used. In particular, nano LC systems have to be treated with great care and require constant maintenance. When using post-column splitting, the increased diffusion can become a problem especially when using systems with very small void volumes. In all experiments ESSI showed better signal intensities than a commercially available, pneumatically assisted ESI source. ESSI does not require heating of the nebulizer gas, which should help to preserve the original structure of thermally unstable molecules. Therefore, ESSI is presented as an alternative to the commercially available heated ESI sources of AB SCIEX, Thermo Fischer, Agilent and Waters. The observed LC-ESSI-MS ion chromatograms are shown to be very stable even when using flow rates higher than 1.0 ml min^{-1}, which could be very suitable for ultra high performance LC, where sample flow rates up to 2.0 mL min^{-1} with backpressures up to 1200 bar are used. Also, a difference in the relative intensities of singly and doubly protonated peptide monomers and dimers was observed between the two ionization methods. The coefficients of determination for the calibration of instrument response for Val–Tyr–Val and Met-Enkephalin showed excellent linearity over a wide concentration range (0.1–100 μM), while ESI results were only linear over a much smaller range (0.1–20 μM). The observed behavior is thought to be caused by insufficient ionization efficiency of solutions above ~20 μM by ESI, exemplifying the robustness of ESSI as an interface between LC and MS.

1. Introduction

Liquid chromatography–mass spectrometry (LC–MS) has become one of the most popular analytical techniques during the last 10–20 years for the analysis of a wide range of compounds [1,2]. The three most widely used atmospheric pressure ionization (API) technique that have been developed for a facile coupling of LC with MS are electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI), as recently reviewed by Kostiainen and Kauppila [2]. Among the most widely used API technique today is ESI. LC–ESI–MS is a highly sensitive and specific analysis method for small polar organic compounds and large biomolecules.

In LC-ESI-MS, water is a poor solvent compared to organic solvents due to its higher surface tension, lower volatility and high dissolving energy, which makes it hard to electrospray aqueous solutions. This is a real drawback if highly aqueous conditions are required for good separation in the LC procedure. A further drawback for LC–ESI–MS is the relatively low maximum tolerable flow rate, which is around 20 μL min^{-1} [3]. This means that conventional LC columns (4.6 mm i.d.) require post column splitting. Higher flow rates, up to 200 μL min^{-1}, can be handled using a pneumatically assisted electrospray, formerly referred to as ionspray, an interface developed by Bruins et al. [4]. Most of the modern ESI interfaces in use today are pneumatically assisted, usually using nitrogen as a "sheath" gas. In 1992, Lee and Henion [5] described a thermally assisted interface capable of operating with flow rates up to

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500 μL min^{-1} using temperatures from 150 to 240 °C. Hopfgartner et al. [6] presented a high-flow LC-ESI-MS interface, which allows for the use of flow rates up to 2 mL min^{-1} by adding a simple liquid shield between the ion sampling capillary and the pneumatically assisted (nitrogen gas pressure 5.4×10^5 Pa) ESI. At the same time Hiraoka et al. [7] presented pneumatically assisted ESI parallel to the ion sampling orifice using 2–2.8×10^5 Pa nebulizer gas (N_2), which gave them the opportunity to accommodate sample flow rates up to 4.4 mL min^{-1}. However, at 4.4 mL min^{-1} the measured ion intensity decreased by nearly a factor of 10. Furthermore, the entire optimization of their setup was done with a much smaller sample flow rate, 100 μl min^{-1}. Sonic spray ionization (SSI) was introduced as an interface for CE-MS and LC-MS in 1994. In this technique, charged droplets are produced without a heating capillary or applying an electric voltage to the capillary tip. A sample solution is introduced through a capillary with a coaxial gas flow. It was shown that charged droplets are produced in the spray at atmospheric pressure at high gas velocities. Gas flow rates up to 6 L min^{-1} could be used, with an optimum at 3 L min^{-1}. It was also found that the ion intensity strongly depended on the gas velocity; an intensity maximum was reached at sonic speed (around Mach 1) [8,9]. A study reported a linear nitrogen velocity between Mach 1 (333 m s^{-1}) and Mach 3 (1000 m s^{-1}) using SSI to couple LC with MS [10]. Ion formation can be explained by the proposed statistical charging model [11]. In 2002, the performance of SSI was compared to the better known ESI interface for LC–MS. Similar results were found and in some cases even better sensitivities for opiate analysis were obtained using the SSI interface [12]. Recently SSI has become commercially available as an interface for LC–MS systems [10,13].

In 1994, SCIEX introduced turbo-ionspray, which is capable of handling a flow rate of 1 mL min^{-1}, by the use of a heated gas beam to improve the desolvation of the sprayed droplets. After 2003, several manufacturers have introduced ion sources for high flow applications using heated desolvation gas, which underscores that there was and still is a need for interfacing to high chromatographic solvent flow rates. A solvent flow rate of 1 ml min^{-1} could be handled with the ESI source offered by Waters, which uses additionally to the nebulizer gas heated desolvation gas to handle this flow rate. SCIEX introduced in 2003 the TurboV source, a further improvement of the turbo-ionspray [14,15]. The HESI II (2008) source is the improved version of the HESI source from Thermo Fisher which uses a nebulization gas temperature of 773 K and an ion transfer tube temperature of 673 K. Using this source, solvent flow rates up to 1 mL min^{-1} can be handled. A flow rate up to 2.5 mL min^{-1} can be efficiently nebulized using the Agilent Jet Stream system (2009) with the aid of a coaxial super-heated sheath gas to create a higher ion density in front of the MS cone. AB SCIEX markets the API 4000 system, which uses a dual heater technology with improved gas dynamics. This ion source is promoted for handling flow rates up to 3 mL min^{-1}.

Electrosonic spray ionization (ESSI) is a new variant of pneumatically assisted ESI, which achieves very efficient nebulization without heating to high temperatures. It was introduced by Takats et al. [16] in 2004 combining a supersonic gas jet (similar to SSI) with ESI. When analyzing protein samples, extremely narrow charge state distributions and sensitivities similar to nanoESI were described. Varying the spray potential from 0 V (pure SSI) to 3.0 kV, the intensity was found to increase by a factor of 2. It was argued that increasing the gas flow rate leads to very efficient solvent evaporation and causes a lower temperature of the spray by adiabatic expansion of the nebulization gas. Moreover, low peak broadening and the observation of one predominant charge state were notable. From their observations it was concluded that proteins are close to a "native-like" conformation in the gas phase when sprayed by ESSI [16]. ESSI-MS has therefore generated interest to study non-covalent protein interactions [16,17].

The aim of this work was to study the figure of merit of ESSI as an alternative HPLC–MS interface to the commercially available heated ESI sources by AB SCIEX, Thermo Fischer, Agilent and Waters. Using higher sample flow rates combined with small diameter columns help to reduce the overall solvent consumption as well as the separation time of an analyses [18]. Therefore, interfaces which can handle high sample flow rates are becoming more interesting. In ESSI, one order of magnitude higher nitrogen pressure (up to 2.3×10^6 Pa) and nebulization gas flow rates are used than typically employed in pneumatically assisted ESI [6]. The higher nebulization gas flow rate produces smaller droplets, which help to get more molecules into the gas phase even when the water content of the solvent is quite high. Moreover, no heating of the nebulization gas is needed, and ESSI is thought to be a much softer ionization source compared to other commercially available ion sources [17].

2. Experimental

2.1. Materials and sample preparation

Water was purified in-house using a Millipore system (Bedford, MA, USA). Acetonitrile (HPLC grade, Sigma–Aldrich, Buchs, Switzerland) and Trifluoroacetic acid extra pure >99% (TFA, Acros Organics, Chemie Brunschwig, Basel, Switzerland) were used to prepare the mobile phases for sample separation. A HPLC standard mixture of the peptides used in this work (0.5 mg Gly–Tyr (G3502) MW 238.2, 0.5 mg Val–Tyr–Val (V8376) MW = 379.5, 0.5 mg Met-Enkephalin (M6638) MW = 573.7, 0.5 mg Leu-Enkephalin (L9133) MW 555.6, 0.5 mg Angiotensin II (A9525) MW 1046.2, was purchased from Sigma Aldrich (Buchs, Switzerland). This peptide mix was dissolved in 1.00 ml (±0.01) water and afterwards used as stock solution. Calibration was performed using seven dilutions of the stock solution (50 μg mL^{-1}, 10 μg mL^{-1}, 5 μg mL^{-1}, 1 μg mL^{-1}, 0.5 μg mL^{-1}, 0.1 μg mL^{-1} and 0.05 μg mL^{-1}) in water/acetonitrile (1:1) containing 0.1% TFA. A 10 μg mL^{-1} Tyr–Tyr–Tyr (Sigma Aldrich, Buchs, Switzerland) solution used for the performance optimization was prepared by diluting 100 μL of a 10^4 μg mL^{-1} Tyr–Tyr–Tyr stock solution in 75:25 acetonitrile/water containing 0.1% TFA.

2.2. Liquid chromatography

All experiments described in this work were performed on an HPLC (Model Shimadzu SCL-10A VP, Duisburg, Germany) equipped with a photo diode array detector (SPD-M10A VP). The LC effluents were delivered by an LC-10AT VP pump which itself was connected to a low pressure mixing valve (FCV-10AL VP). The samples were loaded with an external manual sample injector (Rheodyne, Whitstable, UK) using a 20 μl loop. Method development was done off-line with the photo diode array (PDA) detector set to 220 nm. A flow rate of 1.0 mL min^{-1} was used throughout, unless otherwise noted. The chromatographic separation of the peptide mix was accomplished with a Waters Symmetry300™ C4 column (4.6 mm × 150 mm, 3.5 μm, Waters, Milford, MA, USA) using the following method: starting with 100% A, holding this ratio for 4 min, decreasing in 7 min to 70% A and finally holding this ratio again for 2 min (A = 5:95 acetonitrile/water containing 0.1% TFA; B = 75:25 acetonitrile/water containing 0.1% TFA). The HPLC was controlled by the CLASS-VP version 614_SP1 software.

2.3. ESI source

ESI measurements were carried out using a commercial source (Waters/Micromass Ltd.). The inner diameter of the stainless steel (SS) spray capillary was 75 μm and the spray distance (capillary

tip to the orifice of the Q-TOF) was adjusted to 1 cm. The ESI probe was mounted in the commercially available housing, which fixes the ESI source perpendicular to the MS cone. The tip of the SS capillary was adjusted to protrude 0.5 mm from the nebulizer capillary. The ESI source is capable of handling a flow rate of 1 mL min^{-1}, since it is designed for coupling with standard HPLC systems; for best performance the manufacturer suggests a flow rate of around 200 μL min^{-1} (for further information see supporting information). The nebulization gas flow was set to 2.5 L min^{-1} and the desolvation gas valve was fully opened (∼500 L h^{-1}, 523 K, for further information see supporting information). A capillary voltage of 3.5 kV was applied to the SS capillary. For all comparisons between ESI and ESSI except the experiments where the solvent flow was directly compared, post column splitting was used for the ESI to operate it in optimal conditions.

2.4. ESSI source

ESSI measurements were performed using a modified design of the ESSI source described by Takats et al. [16]. For a detailed description of the ESSI source used for this work see Jecklin et al. [17]. A spray angle of 90° was used throughout, similar to the Z-shaped ESI interface, in order to minimize contamination of the source region. Note that the 90° angle differs from that used in our previous work [17,19]. The spray distance was kept at 1 cm, unless otherwise noted. Nitrogen was used as nebulizing gas at a pressure of 20 bar (5.5 L min^{-1}), unless noted otherwise.

2.5. Mass spectrometer

Mass spectrometric analysis was performed with a hybrid quadrupole time-of-flight mass spectrometer (Q-TOF Ultima; Waters/Micromass Ltd., Manchester, UK, for further information see supporting information) equipped with a Z-spray interface. The instrument was controlled by the MassLynx version 4.0 software. All measurements were performed in positive ion mode. The source temperature was kept at 353 K for ESI and ESSI measurements. The sampling cone voltage was kept at 35 V for all the measurements. The RF1 voltage, the potential applied to the first ion tunnel in the linear flight path before the quadrupole, was varied from 40 to 100 V. The cone gas flow on the other hand was set to ∼1 L h^{-1} for ESI as well as ESSI.

2.6. Safety considerations

Since a huge amount of solvent is being evaporated during the experiments, an additional gas exhaust close to the ESSI source is recommended. Since ESSI generates more noise than conventional ESI, it is recommended to use the interface in a noise reducing housing.

3. Results and discussion

In the first set of experiments, the gas flow rate and spray distances of the ESSI setup were optimized in order to get the highest signal intensity in the absence of the HPLC system. To minimize contamination of the mass spectrometer from the high rate of sample delivery, all experiments were performed using a Z-spray configuration. The summed ion current of Tyr–Tyr–Tyr (m/z 508) from a 30 s acquisition at three different sample flow rates and two ESSI positions as a function of nebulizer gas flow is presented in Fig. 1a. The results were obtained by direct infusion experiments. Three sample flow rates (0.1 mL min^{-1}, 1.0 mL min^{-1} and 2.0 mL min^{-1}) were tested at eight different nebulizer gas flow rates (1.5 L min^{-1}, 2.2 L min^{-1}, 2.8 L min^{-1}, 3.5 L min^{-1}, 4.2 L min^{-1}, 4.8 L min^{-1}, 5.5 L min^{-1}, and 6.2 L min^{-1}). Every point was measured three times by acquiring data for 30 s. The spray position (1–6 cm) was optimized by monitoring m/z 508 $[M+H]^+$ for a sample flow rate of 0.1 mL min^{-1}. The spray distance resulting in the best signal intensity was found to be ∼1 cm. This is in excellent agreement with the data found in the literature where 1 cm has been determined to be the optimal spray distance with various protein samples [16]. When compared to a spray distance of 6 cm, the signal intensity could be improved by a factor of ∼6 (c.f. Fig. 1a). When using a higher sample flow rate the spray plume becomes wider and thus a smaller percentage of the total plume was sampled by the MS cone. This resulted in a decrease of signal intensity. A higher nebulizer gas flow helps to reduce this effect by allowing for better focusing of the spray in the direction of the MS cone.

At a spray distance of 1 cm and a nebulizer gas flow rate of 3.5–4 L min^{-1} the intensity seems to reach a plateau. For a sample

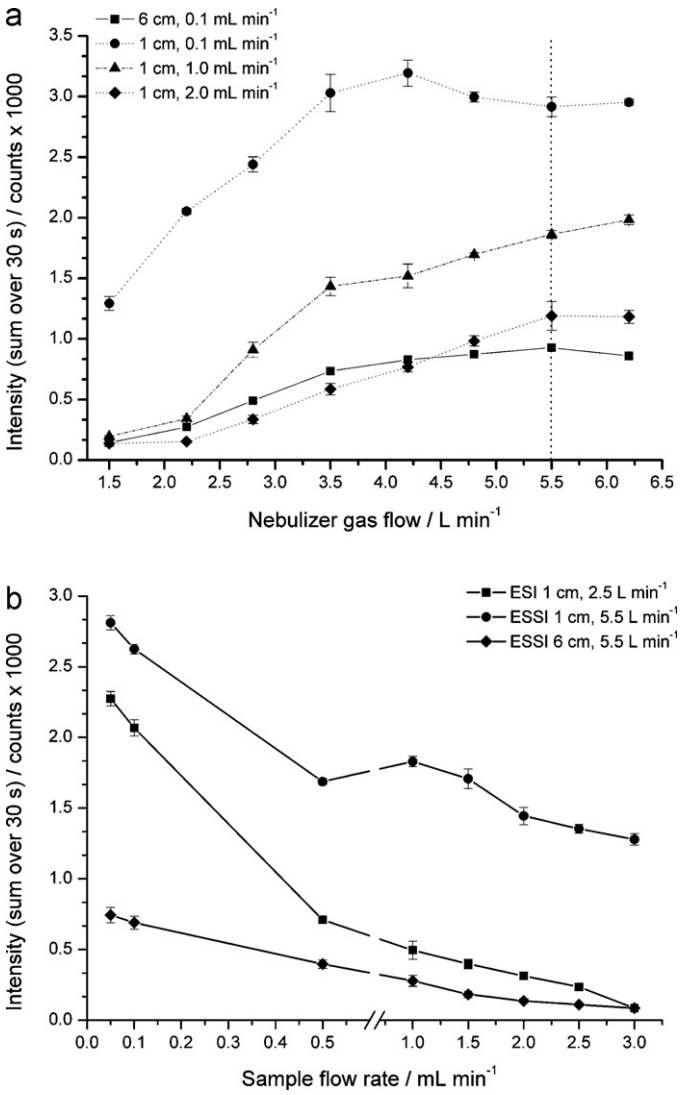

Fig. 1. (a) Dependence of the signal intensity on nebulizer gas flow, for three different sample flow rates and two different distances to the orifice, using the ESSI interface. The sample was delivered to the interface by the LC pump system. Nitrogen was used as nebulizer gas. 30 s of the measured data were averaged (n = 3). The dashed line indicates the nebulizer gas flow rate near the optimum that was used in all subsequent measurements. (b) Signal intensity versus versus nebulizer gas flow for ESI and ESSI, for two distances to the orifice. Sample delivery was achieved using the LC system. The nebulizer gas flow was adjusted by changing the nitrogen gas pressure. 30 s of the measured data were combined (n = 3). The measurements at 6 cm were also performed with ESI but no significant signals were detected.

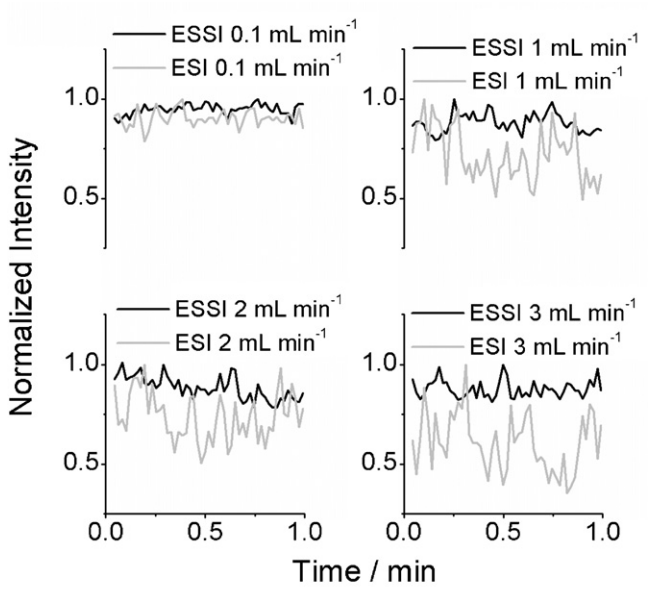

Fig. 2. Normalized signal intensity fluctuations observed with the ESI and the ESSI interface, using four different sample flow rates (0.1, 1.0, 2.0 and 3.0 mL min^{-1}) measured over a period of one minute.

flow rate of 0.1 mL min^{-1}, this plateau was reached at a nebulizer gas flow rate of 3.5 L min^{-1}. For a sample flow rate of 1.0 mL min^{-1} the maximum intensity occurs at the highest nebulizer gas flow tested. Using a sample flow rate of 2.0 mL min^{-1} a plateau appears at 5.5 L min^{-1}. A nebulizer gas flow rate of 5.5 L min^{-1} seemed optimal for all studied sample flow rates, which was the value chosen for all further experiments. However, at a certain point complete desolvation will be reached, as indicated by the plateau. These measurements were repeated for a spray distance of 6 cm. The observed intensity maximum, however, seemed to be reached at higher nebulizer gas flow rates, and the absolute ion current intensity obviously has decreased. The reason for this ion loss is that the spray has a much wider spatial distribution using a spray distance of 6 cm, whereas less ions can enter the MS compared to a spray distance of 1 cm. Therefore a spray distance of 1 cm has been chosen for the ESSI interface. The partial pressure of the solvent in the spray region using ESSI will be rather low and the resolvation of the analyte is slow even though very high flow rates are used. This is thought to be one of the key reasons why ESSI was observed to outperform ESI.

In the next set of experiments, the dependence on the sample flow rate delivered by the HPLC system was studied using the optimal gas flow conditions determined above. This is a key parameter to consider when coupling with LC, in order to figure out which flow rates are tolerated by an ESSI setup. Fig. 1b reports the signal intensity of Tyr–Tyr–Tyr ($m/z = 508$) monitored as a function of sample flow rate. Experiments were carried out with ESI and ESSI, where the latter was operated at two different spray distances (1 cm and 6 cm). Sample delivery by the HPLC pump ensured stable sample flow rates ranging from 0.1 to 3.0 mL min^{-1}. ESI and ESSI both show a decrease of the signal intensity with increasing sample flow rate, however this effect is substantially greater in the case of ESI. For instance, keeping the spray distance fixed at 1 cm, when the sample flow rate was increased from 0.1 to 2.0 mL min^{-1} the ESI generated ion signal decreased by a factor of 30, while the ESSI generated ion signal only decreased by a factor of 2. Moreover, ESSI always yielded higher signal intensities than ESI. This clearly shows that it is quite useful to couple ESSI-MS with an HPLC, especially in the high flow regime.

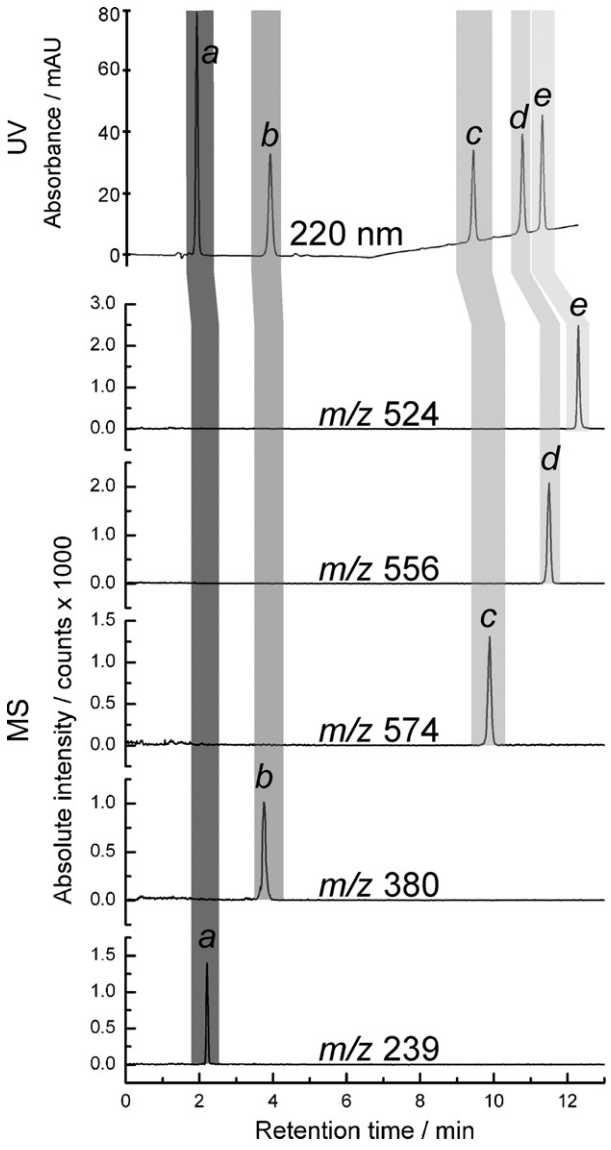

Fig. 3. Separations of five peptides with a concentration of 10 μg mL^{-1}. Injection volume, 20 μL. Peptides: Gly–Tyr (a), Val–Tyr–Val (b), Methionine Enkepahlin Acetate (c), Leucine Enkepahlin (d), Angiotensim II acetate (e). Concentration: a, 42 μM; b, 26 μM; c, 17 μM; d, 18 μM; e, 10 μM.

Using ESSI, quite reasonable signal intensities can be achieved even at a spray distance of 6 cm. At sample flow rates from 0.1, 1.0 and 2.0 mL min^{-1} the signal intensities were within a factor of two of the ESI measurements performed at a distance of 1 cm. This indicates quite a sharp spray plume, which has also been described by Takats et al. [16].

In the following experiment the spray stability of ESI and ESSI at four different sample flow rates (0.1, 1.0, 2.0 and 3.0 mL min^{-1}) was studied. The signal intensities obtained were normalized and are presented in Fig. 2. For ESI, an increase of the sample flow rate leads to a strong decrease in spray stability. In contrast, the spray stability of ESSI is only slightly affected by higher sample flow rates. The very high nebulization gas flow rate seems to provide efficient and stable solvent nebulization, even at high sample flow rates. This result shows that a stable coupling of our ESI source with flow rates » 0.1 mL min^{-1} is difficult, while coupling with ESSI still provides stable signals at higher sample flow rates. Of course, when using high sample flow rates with an ESI source, these instabilities can be compensated for by post column splitting. Splitting the sample flow does have disadvantages, including increased dispersion and

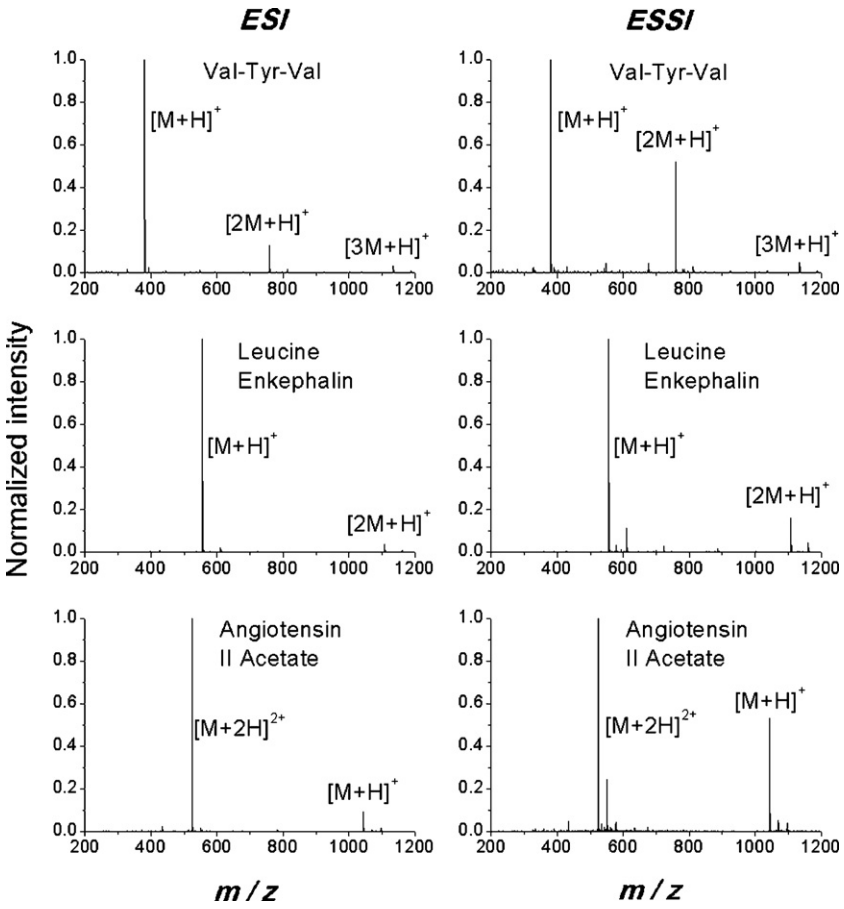

Fig. 4. Mass spectra obtained from the peptieds Val–Tyr–Val (42 μM), Leucine Enkephaline (17 μM), and Angiotensin II acetate (10 μM), using the ESI and the EESI interface.

additional sources of error for quantitative measurements. Using post column splitting usually also results in a larger void volume, since adjustment of the sample flow rate is usually accomplished by using two capillaries with different back pressures or a restriction valve [20]. It is know that the increased void volume also increases the dispersion. Therefore, if high efficiency columns (like in ultra high performance LC) are used, the connection tubes should either be reduced to the absolute minimum in length (small void volume) or, better even, completely eliminated [21]. ESSI allows a simple and stable connection with sample flow rates up to 3.0 mL min^{-1} without any further treatment of the sample flow after the HPLC outlet. Our data suggests that ESSI is a very good choice for interfacing conventional HPLC with MS.

As a further demonstration of the successful use of LC-ESSI-MS, a separation of five small peptides using a C4 column and a solvent gradient is reported. The baseline separation of all five analytes within 13 min was possible using a 1.0 mL min^{-1} solvent flow rate. Note that in order to work at optimal ESI conditions, post column splitting was used (~100 μl min^{-1}). Fig. 3 presents the UV and MS chromatograms obtained from the analysis of the standard peptide mixture (Gly–Tyr, 42 μM; Val–Tyr–Val, 26 μM; Met-Enkephalin, 17 μM; Leu-Enkephalin, 18 μM; Angiotensin II, 10 μM). The offset in the retention time between the UV and the MS traces is due to different void volumes required to reach the respective detectors. Very sharp and intense signals were observed when the ESSI interface with a solvent flow of 1.0 mL min^{-1} was used.

When comparing the ESI–MS and ESSI–MS spectra some major differences can be seen (c.f. Fig. 5). With ESSI, the peptide Val–Tyr–Val forms much more of the singly charged peptide dimer [2M+H^+] compared to ESI. The same was observed for leucine enkephalin. Conversely, angiotensin II acetate shows the most intense signal as doubly charged peptide in both ESI and ESSI. However, using ESSI, a higher singly charged peptide signal compared to the ESI experiment was observed. These results let us conclude that the initial droplet size generated using ESSI must be different compared to ESI. The reason for this could be the higher temperature of the used desolvation gas (nitrogen, 523 K) in ESI, or simply the much higher sample flow rate used in ESSI (1.0 mL min^{-1} compared to <100 μL min^{-1}). It has been reported that ESSI should result in only one dominant charge state when analyzing proteins [10,16]. Since only small peptides were separated here (highest MW = 1046 Da), the use of ESSI only had the effect of decreasing the intensity of the doubly charged species; species with three or more charges were not observed. Cluster formation was also observed, as expected from previous SSI experiments [22]. We think that this clustering in ESSI is a sign of very efficient nebulization.

In order to test the ESSI interface for high flow (1 ml min^{-1}) LC methods, we performed a 6-point calibration for the peptide Val–Tyr–Val and Met-Enkephalin. Using ESI, the same calibration was done by post-column split flow (~100 μL min^{-1}). The calibration was accomplished by adding up all signal intensities that were related to the peptide (singly charged monomer, singly charged dimmer etc.). Injecting 6 different concentration levels for Val–Tyr–Val (130, 26, 13, 2.6, 1.3 and 0.26 μM) and for Met-Enkephalin (87, 17, 8.7, 1.7, 0.87, 0.17 μM) resulted in the calibration curves shown in Fig. 4. The linearity observed in the calibration curves generated by using the ESSI interface was significantly better over the entire concentration range compared to

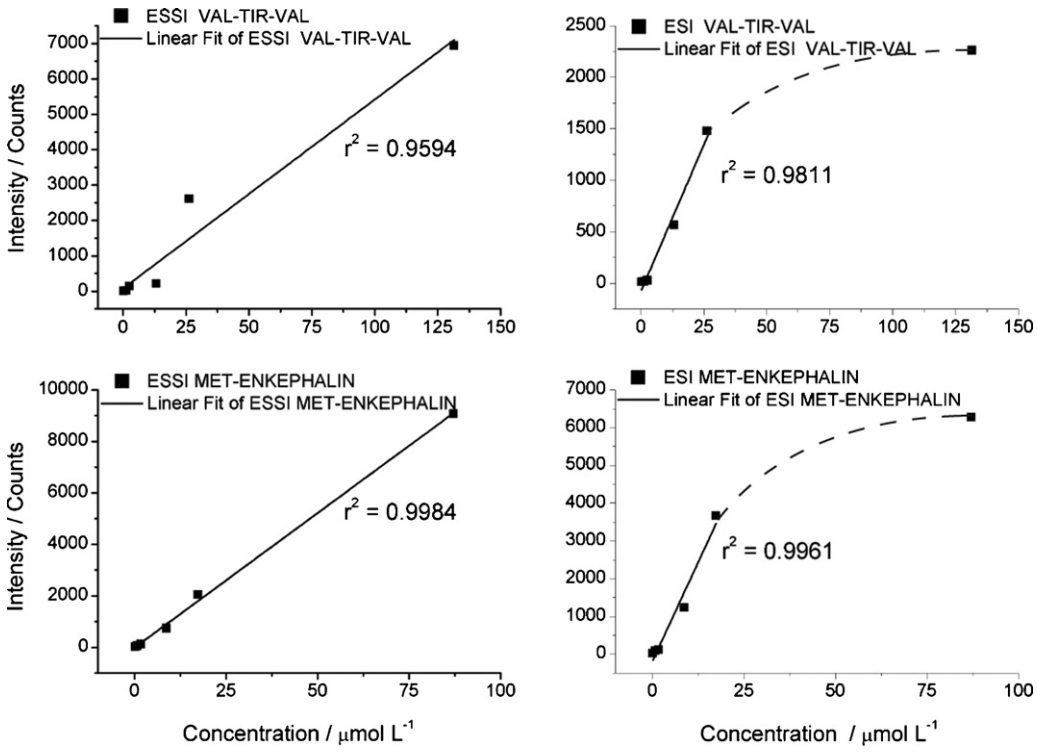

Fig. 5. Calibration plot for the peptides Val–Tyr–Val (Concentrations: 130, 26, 13, 2.6, 1.3, 0.26 μM) and Met-Enkephalin (Concentrations: 87, 17, 8.7, 1.7, 0.87, 0.17 μM) comparing ESI with ESSI as an ion source for the LC. ESI was performed using post column splitting, whereas ESSI was directly coupled to the LC system.

the curves generated using the ESI interface. Coefficients of determinations (r^2) of 0.959 and 0.998 were observed for the peptide Val–Tyr–Val and Met-Enkephalin using ESSI, whereas lower values of 0.759 and 0.837 were obtained by simply placing a line of best fit through all six calibration points obtained for the same two peptides generated by an ESI source. It can, however, be pointed out that ESI shows good linearity up to a concentration of 25 μM. Above this concentration, the ionization efficiency of the ESI interface is probably not sufficient to fully ionize the increasing amount of analyte. Using only the first five points of the Val–Tyr–Val calibration for the ESI interface (r^2 = 0.9811), a linear extrapolation to a concentration of 130 μM would result in a hypothetical intensity of 7300, which is similar to what was observed for ESSI.

It is common to find nebulization gas flow rates in publications about high-flow interfaces for HPLC-MS. However, what really counts when comparing different interfaces is the nebulization gas velocity, not the volume flow. Therefore, we decided to explore the linear nebulization gas flow velocity in more detail. Many different diameters of spray and nebulization capillaries are reported in the literature [6,7,9,16]. The sample capillary of the ESSI source is generally positioned 0.1–0.2 mm outside of the nebulization capillary [16]. Therefore, the calculated nebulization gas velocities in this work are slower at the tip of the sample capillary, since as soon as the nitrogen leaves the capillary end, a continuous deceleration of the nebulization gas takes place. In their review, Covey et al. [23] report gas velocities from 100 to 300 m s^{-1} for the majority of the nebulizers. Kovacs et al. [10], on the other hand, report nebulization gas velocities up to 1000 m s^{-1} (Mach 3) for SSI. The nebulization gas velocity at the outlet of the sample capillary in this work is thought to be in this range. As a simple approximation to be able to compare different ESI interfaces, the gas flow velocity is obtained by multiplying the measured nitrogen flow rate with the area between the inner and the outer capillary. Since, the used ESI interfaces are very similar in their main design a comparison using the easy calculable nebulizing gas velocity seems to be very reasonable. For our ESSI setup, a nebulization gas flow of 5.5 L min^{-1} results in a nebulization gas velocity of 2900 m s^{-1}. For the commercial ESI interface a nebulization gas flow of 5.5 L min^{-1} would result in a nebulization gas velocity of 850 m s^{-1}. The gas flow velocity of the setup proposed by Hopfgartner et al. [6] was calculated to be around 500 m s^{-1}, the one from Hirabayashi et al. [8] around 180 m s^{-1}, and from Takats et al. [16] around 1590 m s^{-1}. Hiraoka et al. [7] observed a rather stable ion signal intensity up to a sample flow rate of 2.3 mL min^{-1}. Higher sample flow rates resulted in a strong decrease of the ion signal intensity, whereas in the work presented here, a slight and continuous decrease of the ion intensity towards higher sample flow rates could be observed. The 2.8 atmosphere back pressure described in their publication should result in a nebulization gas flow rate of roughly 1 L min^{-1}, which for their setup would result in a nebulization gas velocity of 80 m s^{-1}. This is 36 times slower than the nebulization velocity used in our work, which for our interface would lead to poor ion signal stability.

The resulting higher acceleration of the sample helps handling higher sample flow rates, which has been shown in the present work. Due to the high nebulization gas velocity using ESSI the generated droplets are very small. This improves the ionization efficiency, since it is easier to evaporate solvent from smaller than from larger droplets. A key recommendation is therefore to optimize the nebulization gas velocity (rather than simply the gas flow) in the design of new high flow ion sources. A well-designed ion source will improve stability, sensitivity, and could reduce nitrogen gas consumption.

4. Conclusions

Electrosonic spray ionization was applied to the ionization of a HPLC effluent with sample flow rates up to 3.0 ml min^{-1}, and was compared to pneumatically assisted ESI. Using ESSI as an interface offers an easy way to couple of LC with MS while maintaining

high flow rates. Moreover, with ESSI, good spray stabilities can be achieved even when using high sample flow rates. A nebulizer gas flow rate of 5.5 L min^{-1} was found to be optimal for all sample flow rates. Very good coefficients of determination for the calibration of Val–Tyr–Val and Met-Enkephalin were found for ESSI. The main reason for the superior performance of ESSI is thought to be a decrease in the ionization efficiency of the ESI at higher rates of sample delivered as well as higher concentrations (c.f. Fig. 5). ESSI can also be used with standard LC columns, which feature analytical ruggedness, high separation efficiency and can also be used with large injection volumes. ESI is better suited to operate with lower sample flow rates, which is in the flow regime of micro/nano LC systems that are more expensive and usually more difficult to handle than a common LC-system. A standard HPLC-ESI-MS interface requires flow splitting, in which case diffusion can become problematic. Since modern ultra-high performance LC (UHPLC) systems work with very low void volumes (<100 μL), high sample flow rates (up to 2.0 mL min^{-1} with backpressure of >1000 bar) and small diameter columns (very short separation time), they could be coupled directly with the ESSI interface without generating bigger void volume by post column splitting. When using UHPLC rather high sample flow rates are common, which reduce analysis time [18] and the ESSI source could be a good alternative for a high flow MS interface.

At all sample flow rates, ESSI showed higher signal intensities than ESI, even though no heating of the nebulizer gas was used, which could help to preserve to ionize thermally labile substances. Nebulization of thermally unstable molecules using LC with high sample flow rates for separation could benefit strongly from the interface presented here. Furthermore, the ESSI interface provides a higher linear range due to very efficient ionization, well into the mM range.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.04.030.

References

[1] H.J. Issaq, K.C. Chan, G.M. Janini, T.P. Conrads, T.D. Veenstra, J. Chromatogr. B 817 (2005) 35–47.
[2] R. Kostiainen, T.J. Kauppila, J. Chromatogr. A 1216 (2009) 685–699.
[3] C.M. Whitehouse, R.N. Dreyer, M. Yamashita, J.B. Fenn, Anal. Chem. 57 (1985) 675–679.
[4] A.P. Bruins, T.R. Covey, J.D. Henion, Anal. Chem. 59 (1987) 2642–2646.
[5] E.D. Lee, J.D. Henion, Rapid Commun. Mass Spectrom. 6 (1992) 727–733.
[6] G. Hopfgartner, T. Wachs, K. Bean, J. Henion, Anal. Chem. 65 (1993) 439–446.
[7] K. Hiraoka, H. Fukasawa, F. Matsushita, K. Aizawa, Rapid Commun. Mass Spectrom. 9 (1995) 1349–1355.
[8] A. Hirabayashi, M. Sakairi, H. Koizumi, Anal. Chem. 66 (1994) 4557–4559.
[9] A. Hirabayashi, M. Sakairi, H. Koizumi, Anal. Chem. 67 (1995) 2878–2882.
[10] Z. Kovacs, Z. Dinya, S. Antus, J. Chromatogr. Sci. 42 (2004) 125–129.
[11] E.E. Dodd, J. Appl. Phys. 24 (1953) 73–80.
[12] R. Dams, T. Benijts, W. Gunther, W. Lambert, A. De Leenheer, Anal. Chem. 74 (2002) 3206–3212.
[13] T. Arinobu, H. Hattori, H. Seno, A. Ishii, O. Suzuki, Journal of the American Society for Mass Spectrometry 13 (2002) 204–208.
[14] W.M.A. Niessen, J. Chromatogr. A 794 (1998) 407–435.
[15] M.L. Gross, R.M. Caprioli, The Encyclopedia of Mass Spectrometry: Ionization Methods, 1st ed., Elsevier, St. Louis, 2007.
[16] Z. Takats, J.M. Wiseman, B. Gologan, R.G. Cooks, Anal. Chem. 76 (2004) 4050–4058.
[17] M.C. Jecklin, D. Touboul, C. Bovet, A. Wortmann, R. Zenobi, Journal of the American Society for Mass Spectrometry 19 (2008) 332–343.
[18] J. Berry, J.W. Henderson, M. Joseph, LC GC Eur. (2009) 8–42.
[19] D. Touboul, M.C. Jecklin, R. Zenobi, Rapid Commun. Mass Spectrom. 22 (2008) 1062–1068.
[20] E.T. Gangl, M. Annan, N. Spooner, P. Vouros, Anal. Chem. 73 (2001) 5635–5644.
[21] R.P.W. Scott, Liquid Chromatography Column Theroy (1991).
[22] Z. Takats, S.C. Nanita, R.G. Cooks, G. Schlosser, K. Vekey, Anal. Chem. 75 (2003) 1514–1523.
[23] T.R. Covey, B.A. Thomson, B.B. Schneider, Mass Spectrom. Rev. 28 (2009) 870–897.