According to the Woodhull equation, detectable steering effects for both molecular recognition as well as catalysis using synthetic multifunctional pores with $l_A \approx$ 2.7 Å are extremely promising. Imagine the situation with $l_A \approx 17$ Å! To realize this situation, *p*-octiphenyl **1** was designed. In *p*-octiphenyl **1**, peptide strands with 'active' sequences like LRLHL are attached only to the two central arenes. The six peripheral arenes are equipped with 'inactive' arginine- and histidine-free peptides. Self-assembly of *p*-octiphenyl **1** into a β-barrel should then produce a pore with active sites only in the middle. Synthetic efforts toward *p*-octiphenyl **1** and related rods are in progress and will be reported in due course [8].

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Development of a Robust Capillary Electrophoresis–Mass Spectrometer Interface with a Floating Sheath Liquid Feed

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Abstract: The on-line combination of capillary electrophoresis (CE) with electrospray ionisation mass spectrometry (ESI-MS) has attracted major attention for the in-depth analysis of complex samples. However, CE-MS coupling is not straightforward. We present a novel coaxial sheath liquid CE-MS interface, which is robust and can be used for both hyphenated techniques, liquid chromatography-mass spectrometry (LC-MS) and CE-MS, alternatively on the same mass spectrometer. The two separation techniques can be switched within minutes. To obtain a stable ion spray and avoid electrical problems, the CE-power supply is used to produce the potential for the CE separation and the ESI sprayer tip simultaneously. The necessary sheath liquid is delivered by a pump which floats on the ion sprayer potential of the mass spectrometer, avoiding any current flow towards ground. The sole parameter which has to be adjusted to adapt to different CE conditions is a variable resistor. Analytical applications such as peptide mixture analysis and drug screening are presented.

Keywords: Amphetamines · CE-MS · Interface · Peptides · Sheath flow

Introduction

Capillary electrophoresis (CE) has become an essential tool in analytical chemistry. Its ability to separate ions as well as neutral compounds in small volumes with high theoretical plate numbers is indispensable for the analysis of complex mixtures. Theoretical plate counts from 10^4 up to 10^6 can be achieved.

The soft ionisation methods electrospray ionisation mass spectrometry (ESI-MS) and matrix-assisted laser desorption ionisation mass spectrometry (MALDI-MS) have been used routinely as bioanalytical tools in connection with powerful separation techniques. ESI-MS is especially well suited as an on-line detector for separation techniques such as liquid chromatography (HPLC) and CE. This is due to the high sensitivity, high selectivity and the possibility to obtain sequence information of biopolymers or structural details of smaller analyte molecules through MS/MS-methods.

A prerequisite for both the ESI-MS and CE methods is that the analytes have to be

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soluble. Hence, the two methods are comparable and on-line coupling should be relatively straightforward. A first coupling was reported in 1987 [1]. A variety of interfaces have since been described: the liquid junction, the coaxial sheath flow and the sheathless interface, each with its specific advantages and shortcomings [2–5]. Sheathless CE-MS coupling achieves the best sensitivity and separation efficiencies [6][7], however clogging of the usually used micro- or nanospray tips has been reported. On the other hand, operating the interface with an additional sheath flow is less critical in terms of stability and reproducibility of the total ion electropherogram (TIE). Furthermore, it decreases incompatibility problems with the CE buffer and therefore reduces the constraints of ESI-MS in terms of volatile buffer, low salt or low ion-pairing reagents (such as TFA) content. A review of the development of CE-MS interfaces can be found elsewhere [8].

Inherent problems of the CE-MS interface have so far prevented CE-MS from becoming a major hyphenated technique for analytical and bioanalytical laboratories. Common problems are: a) compatibility between the high-voltage power supplies used in the combined instruments; b) unstable ion signals; c) decreased sensitivity; d) the use of the optical detector of the CE is not possible; d) clogging of the ESI-MS nozzle and e) using the same mass spectrometer alternatively for both the LC-MS and CE-MS mode is not possible or the switching is time consuming. The best way to avoid these difficulties might be to obtain the complete CE-MS system from one manufacturer together with the respective interface.

Today, LC-MS systems are present in most analytical laboratories. If a CE instrument is also available, it is often desirable to use the mass spectrometer also for on-line detection on this CE instrument. But often the instruments stem from different manufacturers, and the on-line coupling between MS and CE is not intended or commercially not available. Therefore, an on-line interface has to be built in-house.

This was the situation in our laboratory. The key idea of this work was therefore to find a simple way to couple our Agilent ^{3D}CE-instrument to the Applied Biosystems API 150EX ESI-mass spectrometer, which we usually use as a stand-alone mass spectrometer or as LC-MS detector. In particular, we wanted to develop a CE-MS interface which should meet the conditions as shown in Table 1.

In this paper we describe the development of a novel coaxial sheath flow interface which fulfils the aforementioned requirements. We further present first results obtained with this CE-MS interface.

Table 1. Requirements for the new interface

- Simple and robust
- Switching from LC-MS- to CE-MS-mode should be fast and easy
- No high-voltage problems (sparking, short-circuit)
- Fast change of CE-conditions without major adjustments of the mass spectrometer
- Use of the UV-detector of the CE should still be possible

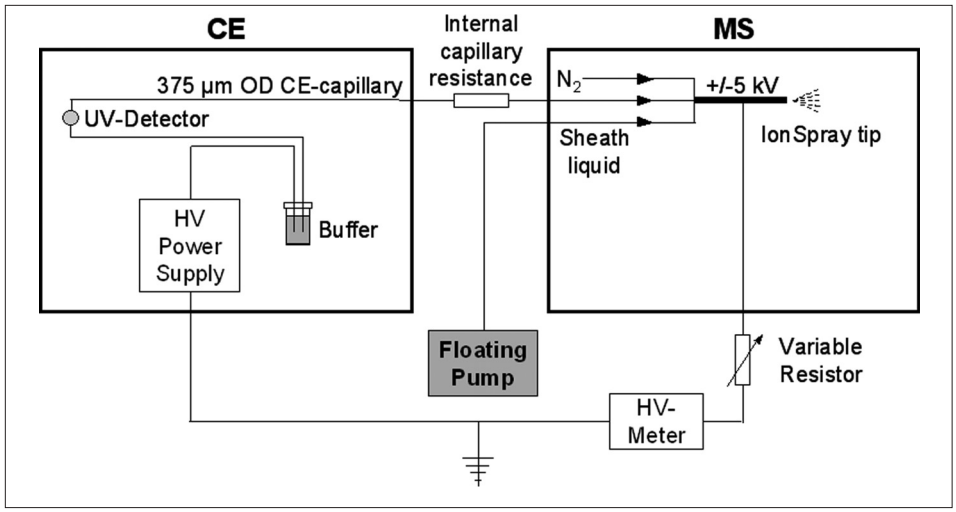

Fig. 1. Schematic diagram of the capillary electrophoresis-mass spectrometer interface with the floating sheath liquid feed. Note that only the CE power supply was needed for CE operation and ion production. The high-voltage meter (HV-Meter) was used for voltage measurements.

Experimental

Materials

Fused-silica capillaries were purchased from Composite Metal Services (Phoenix, AZ, USA). All chemicals used were of analytical or research grade. Acetic acid and ammonium acetate were from Fluka (Buchs, Switzerland). HPLC-grade methanol was purchased from Labscan (Dublin, Ireland). The amphetamine derivative 4-brom-2,5-dimethoxyphenethylamine (2CB) was kindly provided by ReseaChem (Burgdorf, Switzerland). Bradykinin, angiotensin I, angiotensin II, Leu-enkephalin, and Met-enkephalin were purchased from Sigma (St. Louis, MO, USA). Ecstasy pills were received from Eve&Rave (Solothurn, Switzerland).

General Setup

The CE-MS system consisted of a ^{3D}CE System (Agilent Technologies, Palo Alto, CA, USA) and the single quadrupole mass spectrometer API 150EX (Applied Biosystems, Foster City, CA, USA). To allow a fast change between LC-MS and CE-MS mode, two IonSpray source heads (MDS SCIEX, Concord, ON, Canada) with identical connectors for voltage and nitrogen supply were used. The CE capillary was led to the MS after the CE detector cell, which enabled the UV-detector to be used. Thus, monitoring of the CE-separation and quantification using the UV signal was possible under CE-MS conditions. Separations were carried out on uncoated fused silica capillaries of 50 µm inner diameter (outer diameter 375 µm). The overall length of the capillary for CE separation and connection to the MS was 103 cm.

Electrical Layout

A prerequisite of the chosen layout is that one end of the CE power supply has to be grounded. In this case this power supply can be used for both the CE- and the MS-instrument, respectively. This approach avoids any problems such as arcing or voltage mismatch of the often used dual power supply setup for CE-MS interfaces. As shown in Fig. 1, the electrical circuit consisted of the CE power supply which delivered ±30 kV, and two resistors in series: the internal resistance of the capillary and the variable resistor which was used as a voltage divider. To achieve the necessary voltage at the sprayer tip of approximately ±5 kV, the variable resistor had to be adjusted to account for the internal resistance of the CE-buffer in the capillary. Typical values were in the 0.1 to 5 GΩ range. The voltage across the capillary was therefore reduced by the sprayer tip voltage and since the

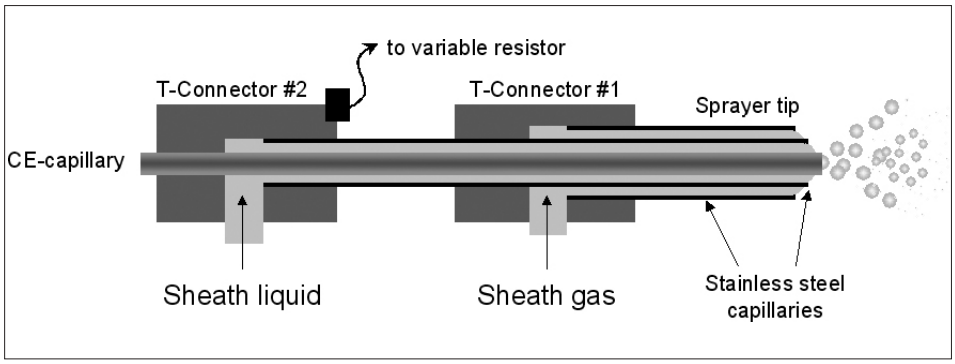

Fig. 2. Schematics of the modified IonSpray ESI probe. The capillaries were used as such, no coatings were applied. The capillaries consisted of fused silica (CE-capillary) or stainless steel.

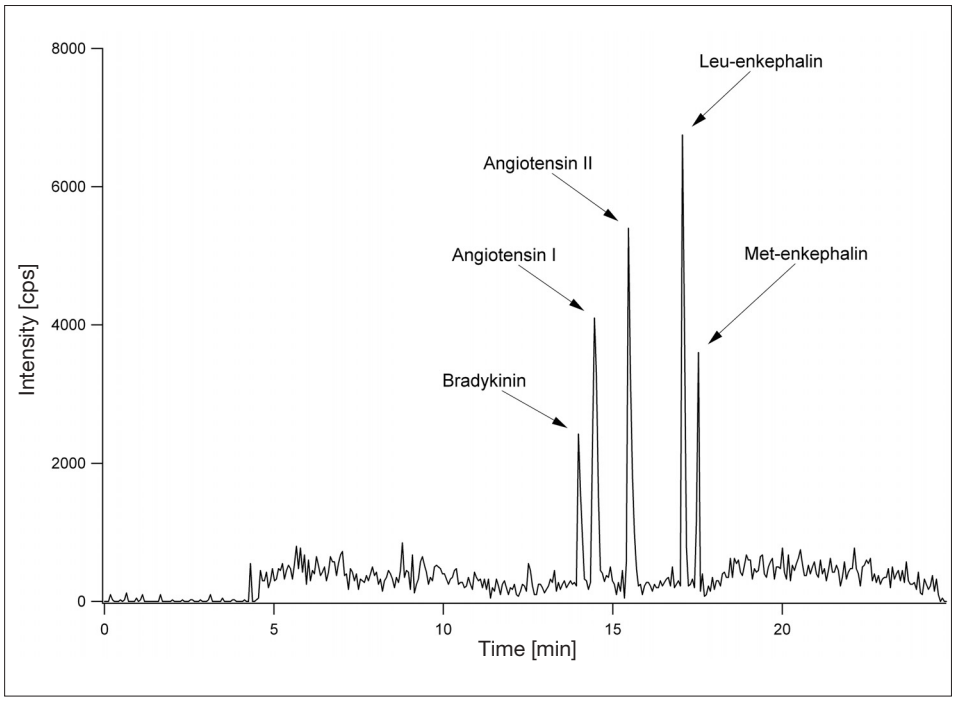

Fig. 3. Selected ion monitoring electropherogram from the analysis of a peptide mixture using a ion spray coaxial sheath flow interface. The mixture was composed of bradykinin, angiotensin I, angiotensin II, Leu-enkephalin and Met-enkephalin (100 ng/μl each). CE conditions: 5 s injection 50 mbar, buffer 10 mM acetic acid, pH = 3.4; fused silica capillary 103 cm × 50 μm × 375 μm (length × I.D. × O.D.); voltage +30 kV (total). Sheath liquid 4 μl/min, 0.5% acetic acid in methanol/water 50:50 (v/v); sheath gas nitrogen (4 bar); variable resistor 5.1 GΩ, current 1 μA. SIM m/z 1061.2, 1047.2, 648.0, 574.7 and 556.6.

power supply of the CE instrument could deliver up to ±30 kV, a voltage maximum of ±25 kV was available for CE separations. The MS internal power source was not needed and it was disconnected from the sprayer tip.

Floating Sheath Liquid Feed

We decided to build a coaxial sheath flow interface to achieve a dilution of the CE buffers to diminish incompatibilities with the electrospray source. The sheath flow delivery system consisted of a syringe and a syringe pump (Precidor, Infors AG, Basel, Switzerland). To prevent current flowing towards ground, the sheath flow system was placed in an isolated plastic box which was floated on the sprayer tip potential. The pump was operated using a battery pack inside the isolated box. Thus, no connection was necessary except for the sheath liquid feed to the sprayer tip.

Fig. 2 shows the setup of the ESI probe. After the detector cell the CE-capillary was led to the mass spectrometer and directly fed into the ESI probe. It replaced the capillary which is usually installed in the IonSpray source. The source was modified to accept a standard fused silica capillary with 375 μm outer diameter. In addition, two stainless-steel capillaries with suitable inner and outer diameters were installed in the ion source as shown in Fig. 2. The dimensions of the inner capillary were 0.53 mm ID and 0.67 mm OD. The size of the outer capillary was 0.75 mm ID and 1.58 mm OD. The sheath liquid was fed through the T-connector #2, while the sheath gas (nitrogen) was applied using connector #1. No coatings on the capillaries were used.

Results and Discussion

Peptide mixture

To test the ability to separate and identify biological compounds in complex mixtures a peptide mixture was prepared and measured according to [9]. The result is shown in Fig. 3.

Except for the ESI probe potential which was delivered by the CE power supply, the ESI-MS was operated as in normal stand-alone mode. The measurement was made in the selected ion monitoring (SIM) mode. All five peptides are well-separated with plate numbers between N = 22400 for angiotensin II and N = 156000 for Met-enkephalin, respectively. Due to the added sheath flow of 4 μl/min, the signal-to-noise ratio was rather low with 1:20. Nevertheless, the interface produced stable and reproducible ion signals as intended.

Amphetamines

The performance of the interface was further examined in qualitative drug analysis. The importance of the analysis of amphetamine and its derivatives is widely recognised for toxicological, clinical, and forensic purposes [10][11]. In particular, the analysis of so-called designer drugs like 3,4-methylenedioxymethamphetamin (MDMA) has become an important topic. Due to the similar migration times for the different amphetamine derivatives, on-line coupling of capillary electrophoresis and mass spectrometry offers the possibility of a simultaneous quantification and identification of these compounds in ecstasy pills.

Many pills which are sold as 'ecstasy' contain other substances besides MDMA. These substances are usually analysed by gas chromatography–mass spectrometry (GC-MS), liquid chromatography or electrophoresis and UV-detection or with immunological techniques. With a CE-MS system, quantification and identification of these substances are possible using both the UV-signal and the mass information. Derivatives with the same molecular mass may show different fragmentation patterns and should therefore be distinguishable.

An example of the analysis of a real sample with our CE-MS interface is shown in Fig. 4, where 4-brom-2,5-dimethoxyphenethylamine (2CB) was found instead of the expected MDMA. The measurement of pills can be done with a minimum of sample preparation. Several

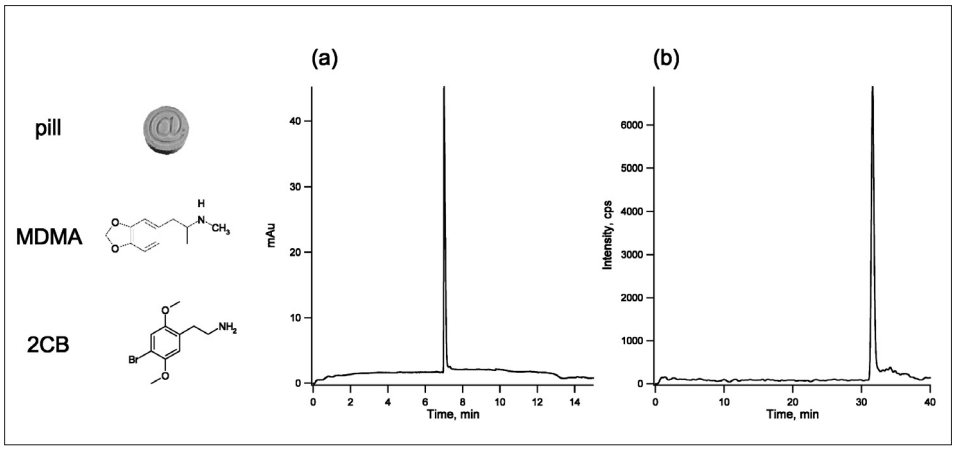

Fig. 4. CE-MS data from the analysis of an ecstasy pill using a ion spray coaxial sheath flow interface. The pill (243 mg) was dissolved in 50 ml water (ultrasonic) and filtered (0.21 μm filter). CE conditions: 6 s injection 50 mbar, buffer ammonium acetate and acetic acid (20 mM each) with a pH of 4.6; fused silica capillary 103 cm × 50 μm × 375 μm (length × I.D. × O.D.); voltage +22 kV (total). Sheath liquid 4 μl/min, 1% acetic acid in methanol/water 80:20 (v/v); sheath gas nitrogen (4 bar); orifice voltage 50 V; variable resistor: 400 MΩ, current 12 μA. a) CE-UV trace 195 nm; b) Selected ion monitoring (SIM) m/z 230.0.

pills were analysed during this work and different amphetamine derivatives could be detected.

The initial test and first analytical results demonstrated that the interface works and that it can already be applied to solve analytical problems. The requirements for the interface as shown in Table 2 could be fulfilled with the described design.

The compromises of the described interface-design are a) it can only be implemented if one end of the CE-power supply is on ground, and b) the sprayer tip voltage is 'lost' for the CE separation potential.

It is obvious that the analytical properties of the interface, in particular sensitivity, limits of detection, tolerance towards different buffers *etc*. have to be tested further.

Nevertheless, the technique might help to bring the CE-MS technique to laboratories which have hesitated so far to use it.

Conclusion

The CE-MS interface described in this article fulfils the requirements for a simple and robust analytical tool. The change from ESI- or LC-ESI-MS mode can be achieved in a few minutes by exchanging the LC-MS-ion spray source with a CE-MS interface and source. The use of only one power supply and the floating sheath flow delivery system diminishes electrical problems. The only adjustment of the CE-MS interface for changed CE conditions are made with the variable resistor.

Table 2. Requirements for the interface and their solutions

Requirements	Solution of this work
Simple and robust interface	Using only the CE power supply for both the CE separation and sprayer tip potential of the ES-MS; use of a sheath flow
Switching from LC-MS- to CE-MS-operation should be fast (within minutes) and easy	Using of two ion source heads for LC-MS and CE-MS operation, respectively
No high voltage problems (arcing, short-circuit)	Use of only the CE power supply for both the CE separation and sprayer potential of the ESI-MS; floating sheath flow feed
Fast change of the CE-conditions should be possible	Only the variable resistor has to be adjusted to new CE conditions
UV-detector of the CE should still be useable	The capillary is connected to the mass spectrometer after the detector cell

Although the sheath flow reduces the sensitivity, the signal-to-noise ratio in the TIE-mode allows qualitative information of CE separated analytes on a routine basis.

Further testing of the interface is underway.

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