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New sheathless interface for coupling capillary electrophoresis to electrospray mass spectrometry evaluated by the analysis of fatty acids and prostaglandins

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Abstract

A new interface for capillary electrophoresis electrospray ionization (CE–ESI) is presented. High voltage is applied at the outlet of the separation capillary by a stainless steel tube, a so called liner, through which the capillary is led. A compensating current between the liquid and the liner is maintained by a natural liquid film, which is built up at the outer surface of the capillary end. Operable potential ranges for differently treated capillary ends have been examined. The liner has been evaluated for the analysis of fatty acids and prostaglandins, all run with the ESI in the negative ionization mode. This simple stainless steel liner should fill the gap, which has prevented CE–MS from being the successful tool, which it has the potential for, namely fast and unattended measurements of analytes in the n*M* range in complex mixtures.

Keywords: Interfaces, CE–MS; Instrumentation; Electrospray ionization; Capillary electrophoresis–mass spectrometry; Prostaglandins; Fatty acids

1. Introduction

The combination of capillary electrophoresis (CE) and electrospray ionization mass spectrometry (ESI–MS) has recently been demonstrated to be a powerful tool for many different applications [1]. Many solutions to the challenge of interfacing these two techniques have been reported, each with its own advantages and drawbacks.

So far, the most popular, universal and robust technique is a sheath-flow interface [2–5], in which a make-up liquid is added to the CE outflow at the capillary end. This was first demonstrated by Smith et al. [2]. The sheath-flow provides the spraying liquid with a high voltage potential. This technique offers advantages such as the ability to perform separations with low or reversed electroosmotic flows and the freedom to modify the matrix to be sprayed. The sheath-flow also increases the total volumetric flow, which reduces the risk of clogging due to precipitation, and the stability of the spray can be enhanced by the use of an assisting nebulizer gas. However, ESI–MS is a concentration sensitive detection method [6], and the sensitivity will therefore suffer from the dilution by a sheath-flow.

A few sheathless CE–ESI interfaces circumventing dilution have recently been presented [7–16]. (i) The simplest possible interface would be to exploit the natural voltage divider between the capillary and the spray, without applying any external high voltage (HV). The voltage at the capillary end and the electrical field in the capillary are mainly controlled by the buffer conductivity together with the applied voltage on the injector side of the capillary [7]. Even

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if this is the most elegant solution, it is restricted operating only in the positive mode, otherwise the capillary surface becomes coated with a positively charged film and the field is reversed. (ii) In order to be able to set the controlling parameters individually – the capillary field, the needle voltage and the buffer conductivity – a second approach is to position an electrode at the very tip of the separation capillary. Two options to do so have been presented, either to coat the capillary end with a metal layer or to attach an electric wire in contact with the liquid at the capillary outlet [8]. The original CE–ESI method, published as early as 1987 [9], used a silver coating to which the high voltage was supplied. A gold coating has also been used but unfortunately, metal coating on glass or silica is mechanically unstable. It does not last for more than minutes or hours, or up to a maximum of a few days if reinforced with SiO_x [10], silanes [11] or a chromium layer below the gold cover [12]. The effort to increase the operable time span involves time consuming surface treatment. (iii) A third alternative is to have a separate spray tip which is attached to the separation capillary, supplying the HV at a junction in between. This can be done by using a stainless steel junction connected to the HV [13], a junction made of porous silica [14] or by inserting an electrode in a hole made in the capillary [15]. However, the delicate manipulations required are difficult due to the small dimensions. For CE combined with UV or laser-induced fluorescence (LIF) detection, the capillary internal diameters are often a compromise between band broadening and sensitivity, but for many of the described CE–ESI–MS interfaces, the compromise is rather between band broadening and practical considerations due to the extremely miniaturized equipment.

The three major limitations of sheath-less nanospray interfaces are the demand for a low surface tension, a liquid of relatively low conductivity [6] and a high enough linear velocity. Early in ESI history, Smith [16] observed that the onset potential of a liquid increases with the square root of its surface tension. He found an upper limit for the surface tension of ~ 0.05 N m^{-1}, above which a stable electrospray in air could not be obtained. The failure to spray liquids with surface tensions > 0.05 N m^{-1} (the surface tension of water is > 0.07 N m^{-1}) was explained by the fact that the necessary field required for the onset of an electrospray exceeds that required for the ionization of air. For ESI–MS infusion work, it was previously shown, that sharpening the spraying needle allowed the applied potential to be decreased. Solvents with higher surface tension could be used, even water in the positive mode, which is a great advantage when, for example tertiary peptide structures are to be studied [17]. Cloupeau [18] could in 1994 theoretically describe the relationship between surface tension and spray capillary radius for achieving spray without a corona discharge. His conclusion was that the capillary outer diameter should be smaller than 12 μm or larger than 360 μm to achieve a non pneumatically assisted electrospray from pure water without corona discharge. The successful combination of tapered and metal-coated capillaries is also the principle on which static nanospray tips work [19].

Vanhoutte et al. compared different spray tips, of which some were internally restricted at the end, for infusion of various methanol-water mixtures with 10 m*M* ammonium acetate as buffer and a flow-rate of 200 nL min^{-1}. If there was no organic modifier in the mixture, they observed a practical lower linear velocity limit at 1000 mm min^{-1} independent of ionization mode. At higher methanol concentrations, significantly lower linear velocities were acceptable [20]. Working close to the lower limit of the operable linear velocity decreases the functional range of the needle potential as well as the stability over time. It also makes the spray vulnerable to small differences between the sample and the buffer matrixes. Running ESI in the positive mode allows a somewhat higher needle potential before false ions appear in the mass spectrum, and the positive mode is consequently somewhat easier to use at low linear velocities or with a buffer which has a high surface tension. Only recently, attempts were made to explain how the linear velocity affects the stability of a dynamic nanospray [21].

One way to increase the linear velocity at the tip of the separation capillary is by decreasing its inner diameter, i.e. an internal restriction. The concept of restricted column ends has been used either to suppress bubble formation in capillary electrochromatography coupled to electrospray ionization

(CEC–ESI) [22] or to achieve a more stable spray and better sensitivity (3–4-fold) in infusion work [12], the latter restriction made with a micropuller. The restriction can lead to clogging of the separation capillary and the analysis time can be prolonged. Wahl et al. [23] invented a method to run sheath-less CE–ESI without organic modifier at only 127 mm min^{-1}. They were successful, not only because they used a capillary which was tapered to 10 μm by HF before silver coating, but also because a gentle flow of sulphur hexafluoride prevented corona discharges at the capillary tip. Thereby they could apply higher needle fields.

The aim of this work was to use a stainless steel liner of the same kind as proved successful in studies with open tubular liquid chromatography (OT-LC) and ESI–MS detection [24]. It is a simple and straightforward way to achieve an independent separation capillary field as well as a spray field without the use of either a sheath-flow, a metal-plated spray tip or a separate capillary tip electrode. Leading the capillary through a piece of a stainless steel tube at high voltage, provides both electrical and mechanical support. It utilizes the phenomenon that the capillary surface is immediately covered with a monolayer of the buffer, which connects the buffer and the power supply. Three methods of capillary end preparation were compared and evaluated for CE work. All studies were undertaken in the negative ionization mode of ESI, making corona discharge/false ion problems easy to detect.

2. Experimental

2.1. Chemicals

Prostaglandins were donated by Britt Yhlen, Astra Hässle, Sweden. Stock standard mixtures of six prostaglandins were prepared in ethanol, and then diluted with a working buffer mixture. Four fatty acids (lauric acid, myristic acid, palmitic acid and stearic acid) were purchased from Sigma–Aldrich. Standard mixtures (1 mg ml^{-1}) of the fatty acids were prepared in tetrahydrofuran (THF), and test solutions were made up by dilution with the working buffer.

2.2. Instrumentation

The instrumental set up of the capillary electrophoresis, the stainless steel liner interface, the high voltage supply and inlet to the mass spectrometer is illustrated in Fig. 1.

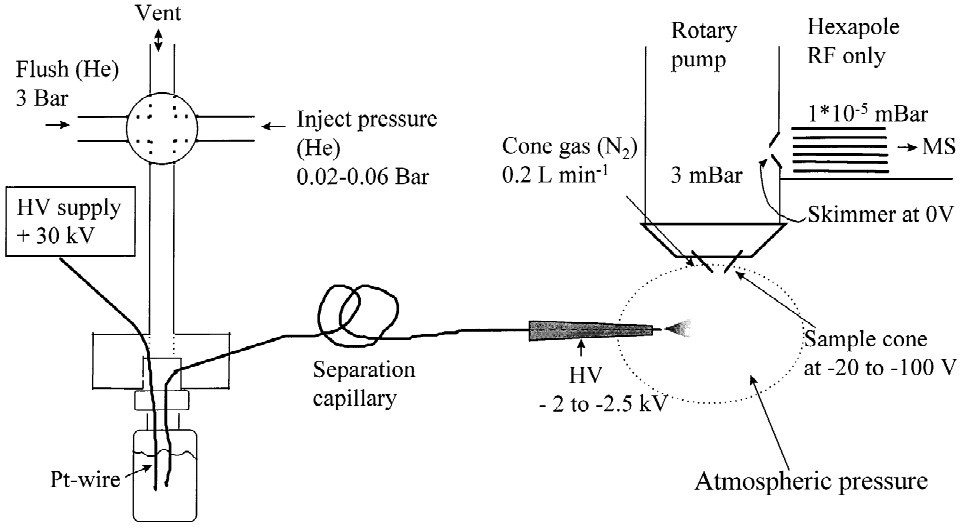


Fig. 1. Schematic picture of the CE setup consisting of the liner interface, high voltage supplies and MS inlet.

2.2.1. Capillary electrophoresis

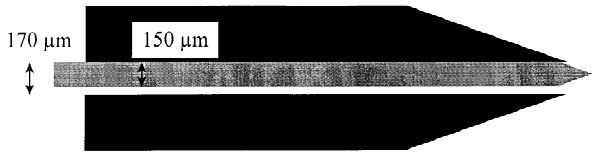
The experiments were carried out using a laboratory-built CE system, where the buffer reservoir carousel was mounted at the front of the mass spectrometer and supplied with HV from a Bertan model ARB 30 (Hicksville, NY, USA) capable of delivering ± 30 kV. Fused silica capillaries were purchased from Polymicro Technologies, USA. In all experiments, 19 μm I.D.×150 μm O.D. capillaries with lengths varying between 29 and 50 cm were used. Prior to separation work, the capillaries were first rinsed for 2 min with 0.1 *M* NaOH and then with buffer solution for a few minutes.

2.2.2. Electrospray interface

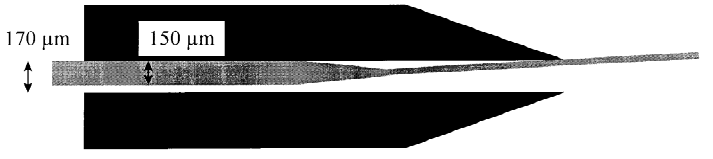
The construction of the interface was based on the liner concept previously used for work with OT-LC coupled to ESI–MS [24]. The outlet of the CE capillary was led through a stainless steel tube, the so called liner, 60 mm×0.17 mm I.D.×1.6 mm O.D. The gap between the fused-silica capillary and the liner was 0–100 μm depending on the tip treatment and the position of the capillary in the liner (Fig. 2). The liner was made by grinding the end of a standard 1/16 in. chromatography steel tube to a sharp end and finally polishing it to remove any sharp edges and obtain a smooth tip (1 in.=2.54 cm). Three different treatments to prepare the capillary end were investigated (all three capillary end treatments are displayed in Fig. 3) and a discussion of each method is presented below.

In method (i) A sharpened fused-silica capillary tip was prepared by wet sanding. The capillary end was drawn through a piece of stainless steel tubing as a support and fastened in place with a piece of tape leaving 3 mm free for grinding. The capillary tip was spun manually at a 20° angle to the sanding disc for 20–40 s. Towards the end of the sanding procedure the capillary was inspected under a microscope to confirm a centered and symmetrical tip. In method (ii) the internally restricted capillary was first sealed by melting over an oxygen/butane microflame, after which it was opened again by sharpening in the same way as above. The aim was to make the restricted part as short as possible. In method (iii) a chemically etched capillary, which was restricted and externally tapered over a longer distance than both i and ii, was drawn with a micropuller and cut at the desired internal diameter. The end was then immersed in 30% hydrofluoric acid (HF) for about 5 min to obtain a smaller O.D. (~ 40 μm).

Mechanical sharpening of fused-silica capillaries and stainless steel tubes was performed on a rotating wet sanding machine (Struers, Denmark) equipped with a laboratory-built second axis capillary holder, rotating along its axis, or, as was later found easier with the fused-silica capillaries, rotated by hand. The liner can be widened at the ''back'' end for conveni-



A) Liner with fused silica capillary sharpened by grinding.



B) Liner with drawn and HF treated fused silica capillary.

Fig. 2. Schematic picture of how separation capillaries with different end treatments are positioned in the liner.

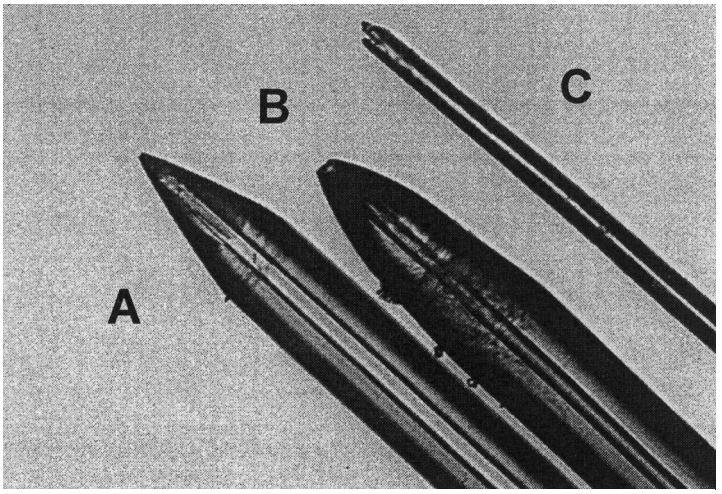


Fig. 3. Photographs of three different 19 μm×150 μm fused-silica capillary end treatments, all of them taken after several days of use. From left to right (A) internally restricted by melting and sharpened by grinding, (B) sharpened by grinding, (C) drawn and HF treated.

ent insertion of the capillary. This was done by electropolishing [25] in a mixture of 30 ml ortophosphoric acid, 53 ml glycerol and 9 ml water at 110°C. The end was submerged for 10 min in the electrolytic bath acting as the anode at a potential of 5 V against a stainless steel cathode.

2.2.3. Mass spectrometry

A Micromass Quattro-LC equipped with a Z-spray ion source was used as detector. The original Micromass dynamic nanospray was modified leaving the *xyz*-board to hold the separation capillary, supply high voltages and allow capillary adjustment for optimal spray characteristics. The ionization block was kept at 80°C. The position of the fused-silica capillary and the spray was observed through a stereo microscope (Nikon SMZ-2T, Japan) mounted at the front of the mass spectrometer, through which photographs of the spray were also taken. Detection was performed in the negative ionization mode by selected ion recording (SIR) of the fatty acids as the anions with m/z 198.9, 227.0, 255.1 and 282.9 for lauric acid, myristic acid, palmitic acid and stearic acid respectively, at a sampling rate of 7 cycles per second. The cone voltage was 60 V. As the studied prostaglandins all have the same molecular mass, m/z 353.2, they were detected as deprotonated molecular ions $[M-H]^-$ and a faster scan rate of 13 scans per second at a cone voltage of 55 V was used.

3. Results and discussion

3.1. Evaluation of liner treatment

Initially we tried to create a CE–ESI interface that was as simple as possible and based on previous experiences with OT-LC–ESI interfacing [24], the first approach considered was to use untreated fused-silica capillaries and liners, i.e., neither tapered nor sharpened.

However, it was discovered that the capillary potential had to be increased beyond the point where false ions appear. Spraying from a straight cut off fused-silica capillary inserted in a sharpened liner gave the same result. This can be explained by the low linear velocity in CE; see Section 3.5 for a further discussion. With the latter combination, slightly pressurizing the anode buffer reservoir increased the velocity leading to a stable spray. However under additional pressure the electroosmotic flow profile will deteriorate, and therefore a better way to stabilize the spray is by sharpening the capillary end. This is because a sharpened capillary end causes a local increase in the electric field at the spray tip whilst the onset field for the corona discharge increases less rapidly, as discussed in several published reports (e.g. [6,18,21,26]) and below in Section 3.2.3. Therefore in conclusion both liners and capillaries should be sharpened, and for

further evaluation of different capillaries a tapered and polished liner was always used.

3.2. Evaluation of capillary end treatments

A set of 30 cm×19 μm non-coated fused-silica capillaries with three different end treatments were tested with various buffers and different capillary fields. To investigate the operable range of linear velocities, the capillary field was varied between 40 and 107 kV m^{-1}. The stability of the spray was quantified as the span between the lowest stable needle potential and the highest potential before glow discharges occurred. The operating parameters investigated were ionic strength of the ammonium acetate buffer and concentration of organic modifier (%, v/v, of acetonitrile) (Fig. 4A–E). The upper limit of the needle potential was measured by increasing the HV, in the negative mode, until a strong signal from $[N_2O_2]$ (m/z 60) appeared instead of the acetate ion (m/z 59) which gives a strong signal of when the spray is stable. The lowest operable needle potential was determined by the disappearance of the signal from the acetate ion (m/z 59). When running in the negative ionization mode, i.e. with the spraying capillary at −2 to −4 kV, there is always a certain potential above which the behavior of ionization changes. This is due to field emission of electrons from the Taylor cone [26]. It has been shown, that an electron capturing gas such as SF_6 in the spray, can suppress electric discharges and the production of false ions, when running in negative mode [3,23]. If the introduction of such a gas was to be used with our interfaces, it would probably increase the operable ranges of linear velocities and buffers.

For all of the three treatments, the operable potential span increased with the content of organic modifier (to a maximum at 50–100%), as well as with the electrical field over the capillary, but decreased with the ionic strength, see Fig. 4A–E.

3.2.1. Capillary end sharpened by grinding only

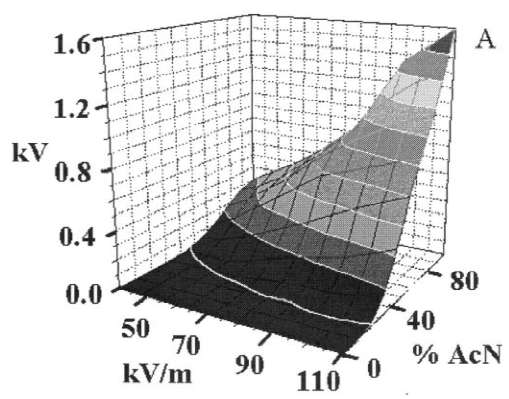
The capillary, which was sharpened only by grinding (i), offered the best overall stability and the largest working potential range as long as the content of acetonitrile in the buffer was higher than 20%. The optimal performance was achieved when the tip of the capillary was pushed approximately 50 μm out of the liner (Fig. 4A and B).

3.2.2. Capillary end narrowed and sharpened by grinding

Unexpectedly, the capillary which was narrowed and sharpened by grinding (ii) showed a similar behavior (Fig. 4E) to the one above, in which there was no restriction of the internal diameter. As can be seen in the photograph (Fig. 3), the length of the restricted zone was made short (~30 μm) in order to minimize the drop in volumetric flow. Fig. 5 illustrates how the average velocity, measured as the capillary length divided by the retention time of acetone, was reduced to about half its original value due to this restriction. The internal diameter at the spray tip decreased from 19 μm to 5–10 μm, which gives a reduction of the nozzle area by a factor of 4 to 16. This means that the linear velocity at the capillary end increased between 2 and 8 times compared to capillary i, at the same time as the volumetric flow-rate decreased to one half.

The work by Vanhoutte et al. [20] showed that when performing nanospray infusion at 200 nl min^{-1} with almost pure aqueous buffer systems, there is a limiting linear velocity of 1000 mm min^{-1} required to achieve a stable spray. When we narrowed the capillary, without changing the outer diameter, to

Fig. 4. Stability tests for the three different capillary tip treatments. Electric field over separation capillary is varied (*x*-axis) as well as content of organic modifier, %(v/v) (*z*-axis) while stability is recorded (*y*-axis). Stability is described as the difference (kV) between the lowest needle voltage for achieving a stabile spray subtracted from the lowest potential when false ions start to occur. In all experiments capillary dimensions were 19 μm×30 cm and no additional pressure at the anode buffer vial was applied. The buffer consisted of acetonitrile (AcN), water and a pH 5.0 ammonium acetate–acetic acid buffer. The following capillary treatments and ion strengths were tested, buffer concentration is expressed as the total content of acetate: (A) Capillary tip sharpened only, 2 m*M* buffer. (B) Capillary tip sharpened only, 10 m*M* buffer. (C) Capillary tip is drawn to about 10 μm I.D. and sharpened by HF, 2 m*M* buffer. (D) Capillary tip is drawn to about 10 μm I.D. and sharpened by HF, 10 m*M* buffer. (E) Capillary tip is internally restricted to about 5–10 μm I.D. and mechanically sharpened, 2 m*M* buffer.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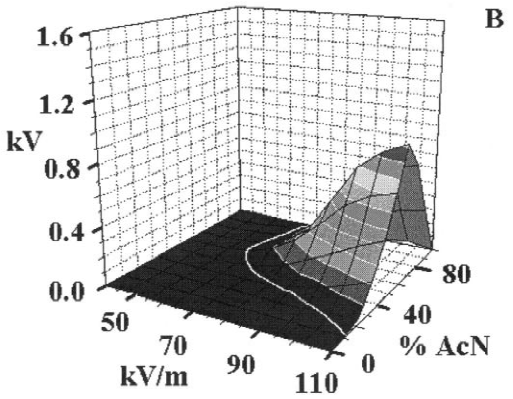
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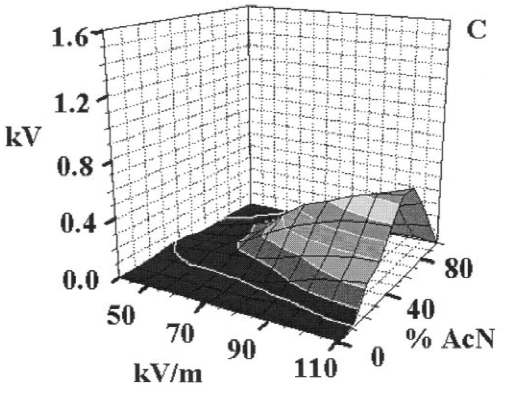
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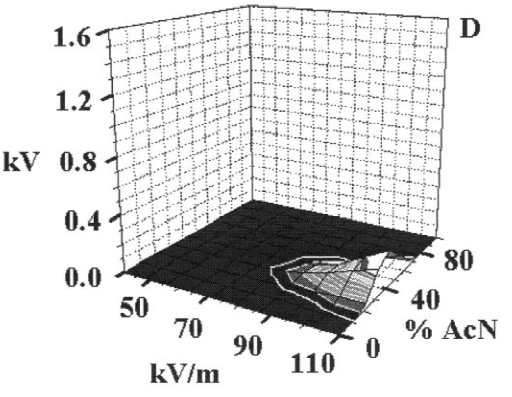
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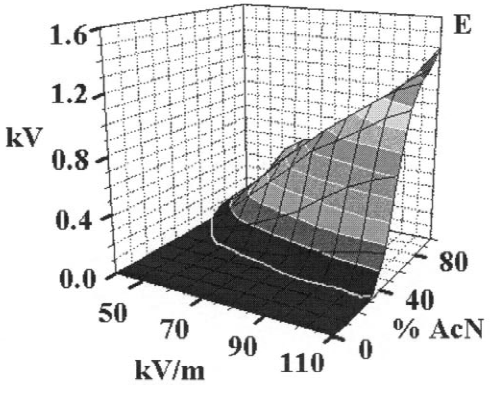
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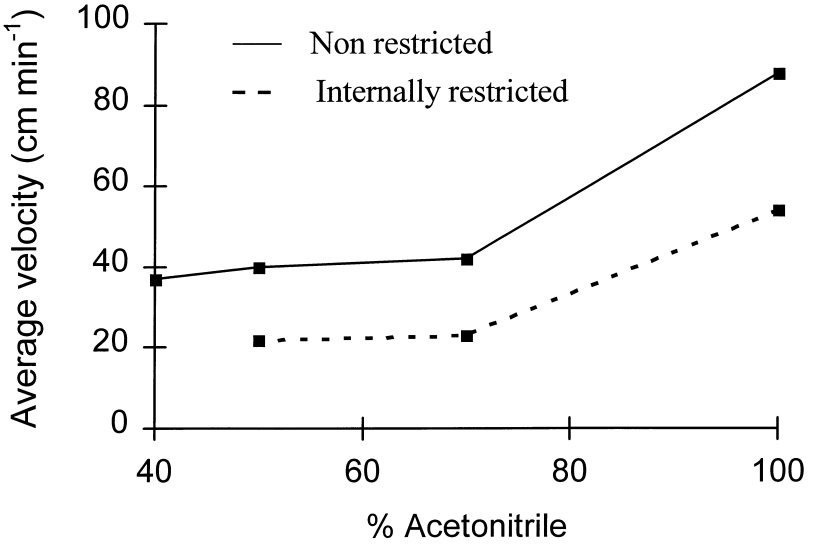

Fig. 5. Plot of linear velocities versus content of organic modifier (acetonitrile) in 2 m*M* buffer (ammonium acetate + acetic acid, pH 5.0). Comparison between two different end treatments: capillary sharpened by grinding only and capillary internally restricted and sharpened by grinding. Total applied voltage over the 30 cm capillary was 32 kV (107 kV m^{-1})

increase the linear flow to about 1000 mm min^{-1} at the tip, we did not experience any stability improvement when spraying pure aqueous solutions. This might be explained by the low volumetric flow (17 nl min^{-1} compared with Vanhoutte's 200 nl min^{-1}). Another possible explanation is that the effect Vanhoutte et al. experienced was an effect of varying both internal and external diameters, thus increasing the field densities at the same time as the linear velocities were increased. In our case, the external dimensions of treatments i and ii were almost identical, giving the same electrical field.

3.2.3. Capillary end drawn and chemically etched

The third treatment of a capillary end (iii), the one which was drawn using a micropuller and etched by hydrofluoric acid (Figs. 4C and D), gave a stable spray when the capillary end was pushed approximately 500 μm out of the liner. It provided a stable spray also at lower concentrations of the organic modifier (acetonitrile) in the buffer, compared with the two former capillaries. Unfortunately, it did not offer as large as operable potential ranges as the other two, and was an overall less stable interface. The working needle potential was −1.8 to −2.0 kV with the HF treated capillaries, significantly lower than the −2.6 to −3.3 kV used with the other two. This discrepancy in needle voltage (V_c) ranges has previously been explained by Eq. (1) [6]. The spray formation depends on the local electric field E (V m^{-1}), which in turn depends on the cross-section radius of the capillary end, and it is E, not V_c, which is the controlling factor of corona onset and spray onset.

$$E = \frac{2V_c}{r_c \ln \frac{4d}{r_c}} \quad (1)$$

where E = the electrical field (V m^{-1}), V_c = applied potential (V), r_c = capillary external radius (m) and d = distance from capillary to counter-electrode (m).

However, the external diameters at the very tips of the two latter capillaries in our investigation, ii and iii must be similar (> 20 μm). We believe the explanation is that Eq. (1) does not take the geometry of the tip into account. For example, the geometry of capillary iii must provide a denser electrical field around the tip as compared with capillary i, because a longer and thinner part of the capillary tip is pushed out from the liner, which affects the

electrical field. This would explain the lower potential span before discharge phenomena appear. Thus, to explain our results, Eq. (1) has to be modified to include the geometry of the tip itself as well as its distance to the voltage supplier, in this case the liner, which both have profound effects on the electrical field.

3.3. Practical considerations of the three capillary tip treatments

The narrowed and grinded end treatment was the most time-consuming alternative. The restriction in the separation capillary should be as short as possible in order to minimize the drop in average linear velocity in the separation capillary. As the inner diameter decreases rapidly at the melted point, the sharpening by grinding demands work under the microscope to obtain a centered hole with the desired inner diameter.

The micropuller drawn and chemically (HF) etched tip is quicker to produce, especially if several are made at the same time. The HF seems to etch off the silica symmetrically, and the important thing is to stop the process in time (after about 5–10 min). Drawbacks are, though, that the narrow section of the capillary is longer, and that the outer diameter of the capillary end is etched off and becomes much smaller than the inner diameter of the liner, which makes it more difficult to establish electrical contact between the liner and the liquid monolayer. This could explain why the stability reached an optimum at about 50% acetonitrile and then decreased again with an increasing amount of acetonitrile. The capillary tip is also quite fragile. Finally, hydrofluoric acid is a hazardous chemical and special precautions must be taken.

The easiest treatment was the sharpening by sanding which takes 1–5 min depending on experience. This was the method offering the most long-term reproducible and rugged capillaries, it also provided the largest operable needle potential range and did not affect the linear velocity. This interface was found to be the most attractive alternative.

3.4. Positive mode considerations

ESI in the positive mode is not as vulnerable to field emissions of electrons causing false ions at high fields. Instead, spray instabilities due to multiple Taylor cones are often observed [6]. Other false ion phenomena can occur at even higher fields in the positive mode, for example formation of water clusters, which are singly charged by a hydronium or ammonium ions [27,28]. This means that lower linear velocities or lower addition of an organic modifier might give a stable spray and good results as compared with the negative mode, due to the possibility of using a stronger electric field. The reason why we have not investigated this further is that Mazereeuw et al. [7] demonstrated an even simpler concept using part of the voltage over the separation capillary to achieve a spray without any additional supply of needle potential. This can be exploited only when running in the positive mode.

3.5. Electroosmotic linear velocity

To translate the capillary field strength to a linear velocity, the electroosmotic flow velocity was plotted against the applied potential with two different buffer systems (Fig. 6). The lowest stable linear velocity, 200 mm min^{-1}, was achieved when acetonitrile–water (1:1) with 2 m*M* ammonium acetate at pH 5 was used as the buffer solution. There is an intermediate range of linear velocities below this in which the spray is only periodically stable. This can be observed as periodic troughs and peaks in the electropherogram arising from droplets built up at the nozzle. As expected, the linear velocity decreases when the ion strength or the water content increases.

Increasing the linear velocity enhances the stability of the spray, at least in the range obtainable in capillary electrophoresis, in our case from 200 to 500 mm min^{-1}. Others working with sheathless CE–ESI interfaces have found that this is close to the operable limits in the negative mode, perhaps with a somewhat larger margin in the positive mode. Increasing the capillary field not only leads to a higher linear velocity, but also faster separations of the analytes and less longitudinal diffusion in the capillary. As shown above, drawing the capillary end to achieve locally higher linear velocities is not a general solution to this problem, at least with the interfaces and the 19 μm capillaries we have used.

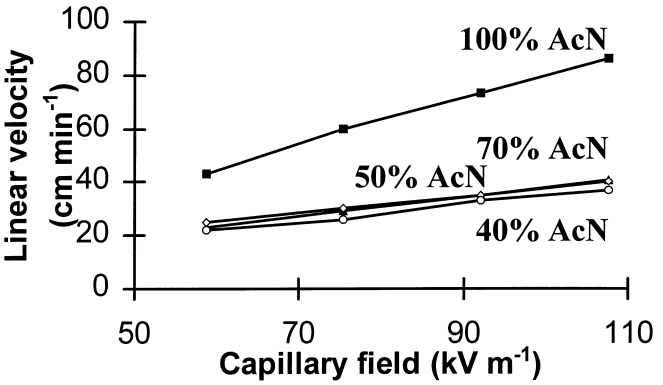

Fig. 6. Electroosmotic linear velocities plotted against applied electric field over a sharpened fused-silica capillary for some of the buffers used. Capillary used was a 19 μm×30 cm treated for a few minutes with 0.1 *M* NaOH, and then rinsed for a few minutes with buffer. Buffer consisted of acetonitrile–water in different v/v mixtures and 2 μ*M* ammonium acetate-acetic acid buffer pH 5.0.

The drawback of a strong electric field is contribution to zone broadening arising from inhomogenous analyte mobility due to temperature effects in the capillary.

The limitation of linear velocity is one of the major disadvantages in sheathless CE–ESI–MS. Natural solutions to this problem would be (1) a strong electrical fields over the capillary, (2) a buffer of low conductivity, (3) a high content of organic modifiers reducing the surface tension and (4) separation capillaries with smaller I.D. to minimize zone-broadening due to heat production. The electroosmotic flow was stable within 1–2%, but this uncertainty can just as well be derived from other sources than the interface, for example temperature effects on viscosity/mobility of the solvent.

3.6. CE–MS performance

To evaluate the effect of the ESI interface on the separation properties, two application studies were carried out. The first example was the separation of four saturated fatty acids. They were chosen because they would either need derivatisation for detection with conventional UV or LIF detectors or gas chromatographic analysis. The fatty acids were separated on a 50 cm×19 μm uncoated fused-silica capillary and detected in the negative mode as $[M-H]^-$ ions by SIR. In the CE capillary they are mainly negatively charged in the chosen buffer, pH 5.2. In the electropherogram in Fig. 7 the peak shapes do not show any band-broadening effects arising from the interface.

In the second example six prostaglandins were analysed (Fig. 8). They are all stereoisomers of one another and are not distinguishable when detected by SIR. Therefore a separation step is necessary. In the literature, no separation method was found that did not involve a derivatisation step. The presence of five chiral carbons in each molecule means that there are many possible stereoisomers of the prostaglandins. Molecular structures of the six isomers used are shown in Fig. 9. The electrophoretic mobilities are very similar, and a 3 min CE–MS run could not separate them all. Four of the six prostaglandins were baseline separated, while the other two co-eluted with two of the others (Fig. 9, 1A with 1B and 3A with 3B). What is needed to separate all of the six stereoisomers is primarily a higher separation efficiency, meaning increased resolution (R_s) and possibly a change in selectivity (α) which could be obtained by addition of other solvents such as dimethylforamide, THF or methanol or by a shift in pH. The resolution would increase with higher mobility of the analytes, longer separation time or narrower peaks. A higher mobility can be obtained by adding charged micelles thereby increasing the charges of the analytes, but also, to a minor extent, by reducing the buffer viscosity or increasing the pH. The only way to increase R_s by longer separation times is, in this special case, a longer separation capillary at the same field strength, i.e., a more

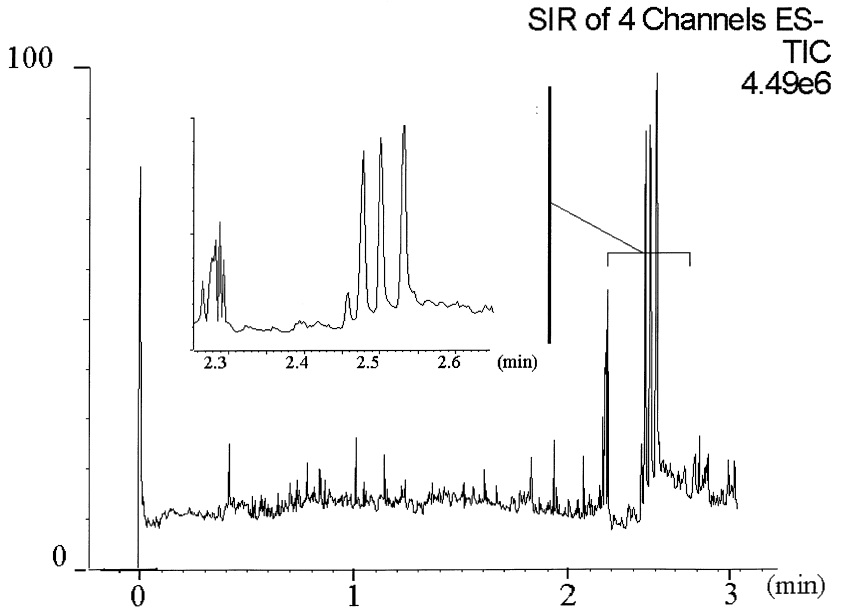

Fig. 7. The separation of four fatty acids (lauric acid, myristic acid, palmitic acid and stearic acid) 20 μg l^{-1} of each, injected 5 s at 2 kPa. Detection was performed by SIR of $(M-H)^-$ with *m*/*z* 199, 227, 255 and 283, respectively, at a sampling rate of 7 cycles s^{-1}. Samples were dissolved in the same buffer as the separation buffer consisting of 2 m*M* acetic acid–ammonium acetate in 50% (v/v) acetonitrile in water at pH 5.2. The largest fatty acid ($C_{18:0}$) is the first peak and the smallest ($C_{12:0}$) is the last. The capillary was only mechanically sharpened by wet sanding, 50 cm×19 μm I.D. and led through a sharpened stainless steel liner. The capillary was positioned 50 μm from the liner. Total applied voltage over the 50 cm capillary was 32 kV (64 kV m^{-1}).

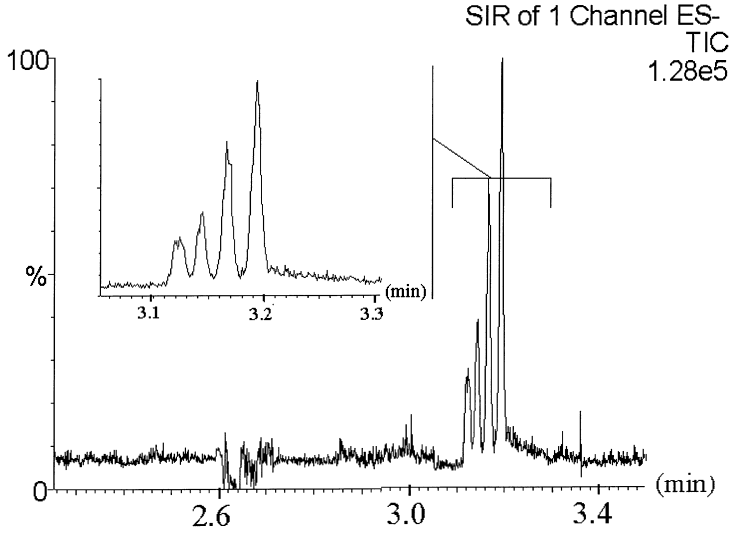

Fig. 8. SIR electropherogram of prostaglandins (see Fig. 9) detected as $(M-H)^-$ all having the same *m*/*z* 353, the sampling rate was 10 scans per second. The concentrations of the six components in the standard-mixture were: (1a) 113 n*M*, (1b) 113 n*M*, (2) 142 n*M*, (3a) 113 m*M*, (3b) 113 n*M* and (4) 708 nM. The sample was injected for 5 s at 2 kPa, which gives between 7 and 9 amol each except for (4), which was 45 amol. Samples were dissolved in the same buffer as the separation buffer consisting of 2 m*M* acetic acid–ammonium acetate in 50% (v/v) acetonitrile in water at pH 5.2. The capillary was mechanically sharpened only, 50 cm×19 μm I.D. The capillary was led through the stainless steel liner and positioned 50 μm out. Total applied voltage over the 50 cm capillary was 32 kV (64 kV m^{-1}).

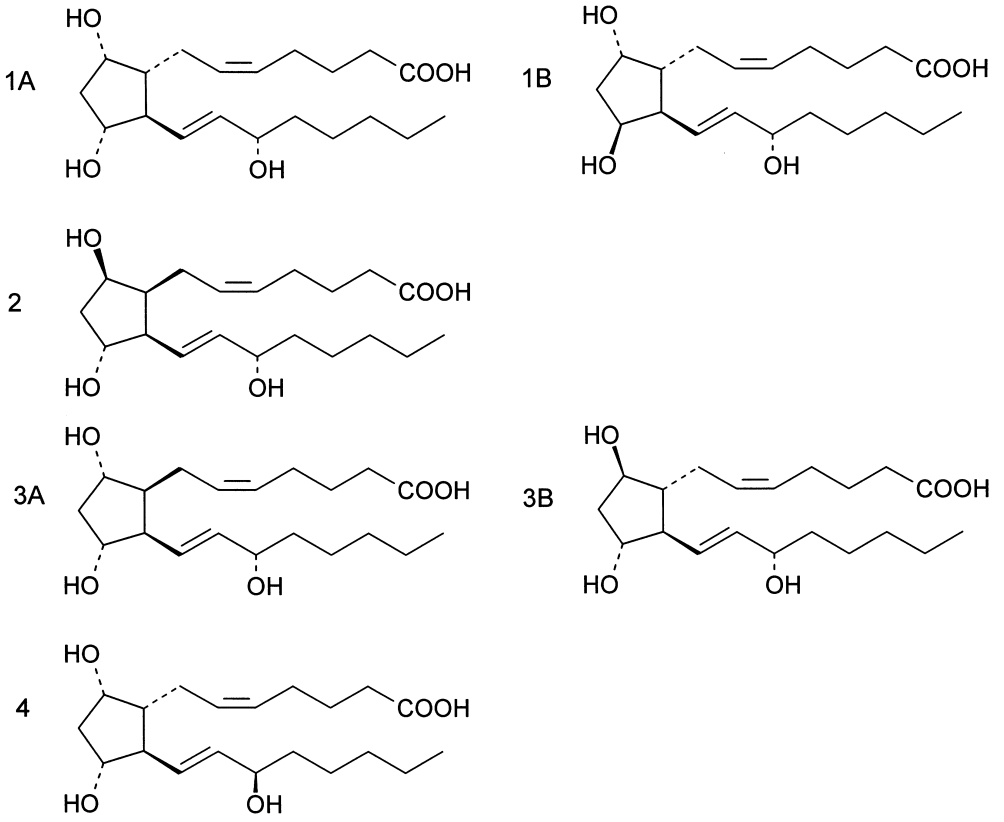

Fig. 9. The six studied prostaglandins. The numbers to the left corresponds to elution order. Peaks 1A and 1B, and 3A and 3B respectively coelute.

powerful HV supply than the one available. If the spray characteristics were not flow dependent, a decreased electroosmotic flow would have increased R_s, as the analytes migrate in the opposite direction of the flow. Finally, narrower peaks would certainly increase the resolution. If detection limits and sample preparation allow smaller volumes to be injected, and if a sample matrix at lower ionic strength was used, this would sharpen the peaks and increase R_s. However, the different contributions to zone-broadening was beyond the scope of this work, and therefore, we have no direct answer to how this could be best achieved. If problems arising from unwanted electrical discharges on the injector side of the system can be solved, it seems favorable to use higher fields together with narrower separation capillaries. It would give a better margin of stability, greater freedom of buffer choice, higher speed of analyses and less zone-broadening.

3.7. Practical considerations

We have observed that increased stability can be obtained by placing the spraying tip very close to the sampling cone in a flow of warm cone gas. In addition, warm gas increases the sensitivity 2–4 times probably by evaporating liquid directly from the Taylor cone (liquid flow-rate ~0.5 nl s^{-1}). Another explanation is that the density of the spray cloud is higher at the sampling cone when the spray needle is placed closer to the cone. The gas is then needed to evaporate the solvent. One has to be careful not to leave the capillary in this position for

hours without the HV connected, as precipitation and clogging might occur.

The capability to form a spray varied somewhat from time to time, partly due to surface effects at the capillary tip and partly due to the Taylor cone itself. We believe this is a minor problem, also with the lipophilic substances run in this study, as long as surface tension is reduced by an organic modifier in the buffer. A much more annoying problem was observed with the internally restricted capillary; clogging of the capillary end if left in air overnight. Even if rinsing with hot water or pressurizing the capillary might help, in our opinion this disqualifies this capillary end treatment. While interchanging between capillaries with fragile end treatments, experiments were done to lead the capillary backwards through the liner to avoid the risk of breaking the treated end. This however introduced dust between the liner and the capillary. The dust usually consists of fine fibers, which (1) increase the risk of glow discharges when soaked in buffer and (2) increase the effective radius (r_c) of the capillary end leading to a lower electric field. To avoid this, one can either rinse the capillary while leading it through the liner or insert the fragile sharpened end first. The non-drawn, mechanically sharpened capillaries are comparatively robust and the risk of breakage is very small.

4. Conclusions

A sheathless electrospray source was built and evaluated as an interface between capillary electrophoresis and mass spectrometry. The capillary end at the detector side was drawn through a stainless steel tube, a liner, to which high voltage was applied. Four fatty acids and six prostaglandins (in the low amol range) were used as test substances. Baseline separation of all the fatty acids and four of the prostaglandins was achieved within 3 min. The sensitivity increases several-fold if the capillary tip is mounted close to and perpendicular to the sampling cone in a counter-flow of heated nitrogen gas. The main contributing factors to the spray stability in the negative mode were investigated for three different capillary end treatments. The conclusions from reproducibility tests are that the best alternative is to (i) use narrow separation capillaries ($\leq$ 20 μm I.D.) in order to reduce zone-broadening from heat production, (ii) sharpen the capillary end by wet sanding, (iii) lead the capillary end through a sharpened liner and let it extend 50 μm, (iv) use a low conductivity buffer which contains at least 20–30% (v/v) of organic modifier, and (v) apply a CE field over the separation capillary which is at least 1 kV cm^{-1}. Thereby low enough surface tension and high enough electroosmotic flow is obtained, a prerequisite for fast, unattended measurements of complex mixtures of analytes in the nmol l^{-1} range.

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