A nanoLC-MS-based platform for peptide analysis
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Characterisation of a nanoelectrospray interface

2.1 Introduction

Nanoelectrospray (nanoESI) was developed by Wilm and Mann [1,2]. Though nanoESI first referred to an off-line application, the need for very sensitive analysis in proteomics led to the coupling of nanoliquid chromatography (nanoLC) to MS using nanoESI. In nanoESI, the spray voltage can be applied on a conductive layer on the outside of the emitter [2,3] or directly to the liquid to be sprayed [4–6]. Both set-ups present advantages and drawbacks. The metal coatings used as conductive layer deteriorate following corona discharge [7]. Moreover, they adhere poorly to silica and often show limited lifetime [8]. When applying the voltage directly to the liquid to be sprayed in a so-called ”liquid-junction” set-up, non-coated silica emitters are used [4–6]. The emitters used in liquid-junction interfacing are easier and cheaper to prepare. However, they require a higher voltage to produce a stable spray than coated needles, due to the significant voltage drop through the solvent, due to solvent resistance. The different means to apply voltage to the sample solvent are likely to influence the quality of the spray and the resulting spectra. Additionally, eluent flow rate and composition, as well as the internal diameter of the nanoESI tip, influence the stability of the spray [9] and its morphology [10]. It was found that, at certain solvent compositions, signal intensity drops dramatically. Such a decrease is likely to happen at various mobile phase
compositions in nanoLC-ESI-MS as well. The use of tapered gold-coated tips ensures efficient ionisation over a much wider range of mobile phase composition [3]. At very low flow rates (<50 nL/min), ionisation suppression is greatly reduced compared to that at higher flow rates [11]. Emitters with a tapered end of smaller ID and thinner walls gave the best results in terms of sensitivity and stability at such low flow rates. A reduction in flow rate was found to be concomitant with a decrease in the optimal spray voltage [12]. With respect to the resulting spectra, parameters such as tip diameter, flow rate, analyte concentration and solvent composition can all affect the ions observed and their relative intensity [13]. In addition, positioning of the nanoESI tips, the type of emitters and their dimension are also of importance. Consequently, in order to characterise a nanoESI interface for proteomic analysis, it is necessary to investigate the impact of varying the aforementioned parameters on the spray and the spectra. In this study, our home-built nanoESI interface was characterised in order to allow selection of optimal experimental conditions for further developments of the nanoLC-MS-based platform.

2.2 Materials and methods

2.2.1 Materials

Formic acid (98-100% pure) was purchased from Merck KGaA (Darmstadt, Germany) and acetonitrile (HPLC Supra-Gradient grade) from Biosolve B.V. ( Valkenswaard, The Netherlands). Bovine insulin (≥ 27 USP units per mg) was obtained from Sigma Aldrich (Zwijndrecht, The Netherlands), SSI stainless steel (SS) union (1/16” tubing OD, 0.015” bore), 1/16” SS nuts and 1/16” SS ferrules and 1/16” PEEK tubing (0.02” ID) from Supelco (Zwijndrecht, The Netherlands). PEEK microCross (38 nL dead volume) and microtight PEEK tubing sleeves (395 µm ID) were purchased from Upchurch. Fused-silica capillaries (100 µm ID, 360 µm OD) were from Composite Metal Service (Ilkley, UK), and tapered fused-silica nanospray needles (360 µm OD, 75, 50 or 20 µm ID and tapered to 15, 8 or 5 µm ID, respectively) from New Objectives (Woburn, MA, USA). Nanospray (nanoESI) needles (also referred to as tip, spray tip, or emitter) were either platinum-coated at the tapered or distal end, or uncoated. To differentiate between the three kinds of nanoESI needles, we will refer to them as fully-coated for the tapered-end platinum-coated needle, distal-coated for the distal-end platinum-coated emitter, and bare-silica for the non-coated spray tip. When referring to a nanoESI tip by its ID, the ID will be the one at the tapered end of the emitter (i.e. 15, 8 or 5 µm.
ID. Nanotips may require “opening” by gently tapping the tapered end of the tip against the end plate of the MS [2]. Their ID at the tapered end was thus larger than specified by the company.

2.2.2 Sample preparation

Solutions of 600 nM bovine insulin (BI) were prepared by sequential dilution in solvent containing water, acetonitrile (ACN) and formic acid (FA). The amount of water, ACN and FA in the solvent were varied in accordance with the parameter that was investigated (i.e. %ACN, pH). The solvent composition is explicitly given in the relevant parts of the ”Results and Discussion” section.

2.2.3 Set-up

Different means of connecting a transfer capillary or a liquid chromatography column to a nanoESI emitter were investigated (Fig. 2.1). The different nanoESI interfaces were mounted on a home-built micrometric table placed in front of an LCQ ion trap MS (Thermo Electron, Breda, The Netherlands). The micrometric table allowed the accurate positioning of the nanoESI tip with respect to the inlet of the MS, which is in the center of the MS heated capillary. Electrical contact was made by applying the high voltage on the outside of a platinum-coated nanoESI tip (cf. scheme D Fig. 2.1) or directly to the liquid to be sprayed (cf. schemes A,B & C, Fig. 2.1), in which case the interface is referred to as a ”liquid junction”. Most experiments described here were performed using the set-up shown in schemes A and B with a bare-silica needle, unless otherwise stated.

Different parameters are likely to influence the quality of the spray and the spectra. These include spray voltage, distance between nanospray tip and MS inlet, mobile phase composition (% organic modifier and acid), dimensions of nanospray tips and possible coating.

The coordinates of the nanospray tip were varied so as to investigate the impact of the distance between the nanoESI tip and the MS on the MS signal. For every set of coordinates, a stable spray was obtained by adjusting the spray voltage, and spectra were recorded for 1 min. The spectra were averaged over the whole sampling period (1 min). The intensity and signal-to-noise ratio (S/N) of the resulting spectra were used to compare the influence of the parameters investigated in this study. In the following paragraphs, S/N refers to the intensity of the MS signal with respect to the spectral noise. S/N in the spectral domain is important for the discovery of biomarkers by nanoESI-MS. The S/N in the time domain will
be of greater importance than the S/N in the spectral domain for monitoring of peptides, though the former is influenced by the latter.

Figure 2.1: Different means of connecting a transfer capillary or a nanoLC column to nanoESI tips. A and B are views of a "liquid junction"-type connection making use of an SSI stainless steel union (1/16” tubing O.D., 0.015” bore). High voltages were directly applied to the stainless steel connection. In C, electrical contact is also made through a "liquid junction" within the PEEK microcross (38 nL dead volume). The microcross allowed the addition of a make-up liquid or of a standard solution. In D, ionisation is achieved by applying the high voltage on the outside of a fully-coated nanoESI tip. The capillary or nanoLC column is butt-connected to the nanoESI tip using a Teflon sleeve ensuring minimal dead volumes.

The procedure was the same for every parameter that was studied (angle of the nanospray tip with the MS heated capillary, mobile phase composition,
dimensions of nanospray tips and type of coating if present). Since the aim of this study was to gain a better understanding of the nanoLC-MS interface and the optimal conditions under which it should be used and not to acquire analytical data per se, experiments were not replicated.

### 2.2.4 MS settings

Optimisation of the MS transmission parameters (capillary voltage, tube lens offset, voltage on the octapoles and the interoctapole lens) was performed using the pneumatically-assisted electrospray interface and software provided with the LCQ ion trap MS (Thermo Electron, Breda, The Netherlands). A solution of 6 µM BI in 0.1% FA in water:ACN (1:1) was infused through the ESI interface at 5 µL/min. The ion-transmission parameters are independent of the flow rate used during tuning of the MS, but are characteristic of an analyte. Later tuning/control of the MS settings was performed using a 600 nM solution of BI in 0.1% FA in water:ACN (1:1) infused through the home-built nanoESI interface at 200 nL/min. Tuning of the different transmission parameters was done by varying the parameters manually and subsequent visual inspection of the resulting MS spectra. The optimum parameters are summarised in Table 2.1. Clear protein spectra with little spectral noise could be obtained using the MS settings shown in this table. The high value found for the optimal capillary voltage for analysis of BI is likely to result in in-source fragmentation in the case of peptide analysis, and could prove detrimental to sensitivity and reproducibility [14, 15]. For the mass spectrometric analysis of substance P, a neuropeptide of importance in many brain pathologies, even the lowest capillary voltages resulted in in-source fragmentation (data not shown). Regular tuning is necessary in order to preserve good sensitivity. Moreover, the optimal MS settings should be investigated whenever analysing a new compound [16].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heated capillary voltage</td>
<td>&gt;0 V</td>
</tr>
<tr>
<td>Tube lens offset</td>
<td>15 to 50 V</td>
</tr>
<tr>
<td>2nd octapole</td>
<td>-4.5 to -9.0 V</td>
</tr>
<tr>
<td>1st octapole</td>
<td>-3.0 to -4.5 V</td>
</tr>
<tr>
<td>Interoctapole lens</td>
<td>-15 to -40 V</td>
</tr>
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**Table 2.1:** Summary of the optimum transmission parameters for bovine insulin. Clear protein spectra with little spectral noise could be obtained using these MS settings.
2.3 Results & Discussion

2.3.1 Angle of the nanotip with respect to the position of the heated capillary

Positioning the nanoESI tip at an angle with respect to the longitudinal axis of the heated capillary is a generally accepted approach to improve the S/N ratio by decreasing the number of neutral species entering the MS. Alternatively, nanoESI interfaces can be positioned on-axis and very close to the MS inlet to transfer as much as possible of the sample into the MS in order to reach a lower limit of detection (LOD).

To this end, a 600 nM solution of BI in H2O:ACN:FA (70:30:0.1) was infused through an 8-µm-ID uncoated nanospray tip at a flow rate of 200 nL/min using a 250-µL Hamilton syringe and the syringe pump of the LCQ MS. The tip was either in line or at a 20° angle with respect to the heated capillary. The coordinates of the nanospray tip were varied so as to investigate the impact of the distance between the nanoESI tip and the MS on the MS signal. Over the whole set of coordinates, the spray voltage was set between 1.0 and 3.4 kV. The further the tip was positioned from the heated capillary, the larger the voltage required to obtain a stable spray. The electric field followed the same trend though it quickly levelled off with distance (Fig. 2.2). The optimal spray voltages appear to be larger when the nanospray tip was at a 20° angle with the heated capillary than when it was positioned on-axis. It was necessary to “open” nanotips by gently tapping the tapered end of the tip against the end plate of the MS [2]. A simple explanation might therefore be that this operation has led to different geometrical characteristics of the tips and resulted in the observed differences in spray voltage. However, this would need to be confirmed with further experiments. Though positioning the interface on-axis gave higher intensities for both BI$^{5+}$ and BI$^{4+}$, operating the interface at an angle seems to result in larger S/N values. When the tip was positioned on-axis and very close to the MS inlet, the sample was sprayed directly into the MS inlet, which would explain the larger intensities. However, when the nanospray tip was positioned further away from the heated capillary, the diameter of the spray was too large for all the ions to enter the MS. Therefore, the increase in signal intensity when the tip was positioned further away from the MS inlet probably resulted from more efficient ion evaporation. Chemical noise results from collisions of charged analytes with neutral species in the MS. When the tip was positioned at an angle, fewer neutral species could enter the MS, which resulted in lower chemical noise and thus larger S/N.
Concluding, positioning the nanospray tip at a 20° angle with respect to the heated capillary is favourable in obtaining spectra with a low background level, i.e. when screening samples for novel biomarkers. However, when monitoring known species, the interface should be positioned axially to achieve maximal intensity for a single m/z and thereby sensitivity.

![Graph](image)

**Figure 2.2**: Variation of the optimal spray voltage (diamonds) and electric field (triangles) with the distance at which the nanoESI tip was positioned. A 600 nM solution of BI in H2O:ACN:FA (70:30:0.1) was infused through an 8-µm ID uncoated nanospray tip at a flow rate of 200 nL/min using a 250-µL Hamilton syringe and the syringe pump of the LCQ MS. The tip was positioned at a 20° angle with respect to the MS heated capillary. The lines indicate the general trend for the variation of the spray voltage and the electric field.

### 2.3.2 Impact of the percentage acetonitrile (ACN)

To study the impact of an organic modifier on signal intensity, two solutions of 600 nM BI were prepared in H2O:ACN:FA with two different ratios. The percentage FA was fixed at 0.1%, but the percentage ACN was set at either 30 or 60 % (H2O at either 70 or 40%). Both solutions were infused through an uncoated 8-µm-ID nanoESI emitter at 200 nL/min using a syringe pump. Optimal spray voltages were much lower for the 60% ACN solution (0.9-1.2 kV) than for the 30% ACN solution (1.2-3.4 kV).
Figure 2.3: Influence of the concentration in FA in the sample solvent on the spectra. Top: 0.1% FA. Middle: 0.5% FA. Bottom: 1.0% FA. All three solutions contained 600 nM BI and were prepared using solvents containing 40% water and 60% ACN. The sample solutions were infused through a non-coated 8-µm-ID nanoESI tip (at a 20° angle with respect to the MS heated capillary) at 200 nL/min using a 250-µL Hamilton syringe and the syringe pump of the LCQ MS.

The lower surface tension and easier evaporation of the solvent containing...
60% ACN are responsible for the much lower spray voltages necessary to obtain a stable spray as compared to the solvent containing 30% ACN. When infusing solutions with little ACN, the spray voltage must be carefully adjusted in order to obtain a stable signal. Spray voltages which are too low result in the solvent sputtering from the nanospray emitter [10]. Spray voltages which are too high, on the other hand, cause the spray geometry to change [10] or induce arcing [7].

In contrast to a solvent containing little ACN, solvents containing a large percentage of organic modifier allow the spray voltage to be set at a much broader range of values while retaining a stable spray and a good S/N. It has been reported that the addition of glycerol and m-nitrobenzyl alcohol to the analyte solutions to be sprayed dramatically increased both the maximum charge state and the signal intensity of the proteins observed [17]. In our study, the amount of ACN did not have any bearing on the charge state, as no significant difference was observed between the intensity ratio for BI$^{4+}$ and BI$^{5+}$ obtained with either 30 or 60% ACN. However, when the sample solution was prepared in 10% ACN (cf. Fig. 2.4 in paragraph 2.3.4), the BI$^{6+}$ ion was also observed, whereas it was not visible at an ACN percentage of 30 and 60%. Probably one of the six nitrogen atoms of BI that are likely to be protonated must have been shielded from the protons of the surrounding solvent, either because of the forced folding of BI or aggregation at higher % ACN.

### 2.3.3 Influence of the formic acid concentration

Varying the pH (through a change in the FA concentration) is a common approach to improve chromatographic efficiency or to vary the selectivity of a chromatographic column. In addition, a change in pH will affect ionisation and therefore the charge state distribution. Sensitivity might also be affected by changes in pH. It is therefore necessary to find the optimum FA concentration. To study the effect of pH on the MS signal, three solutions containing 600 nM BI were prepared using solvents containing 40% water, 60% ACN and concentrations of FA of 0.1, 0.5 and 1.0 %. The resulting pHs are 2.7, 2.5 and 2.2 respectively. The sample solutions were infused through an uncoated 8-µm-ID nanoESI tip at 200 nL/min. At 0.1 % FA, three distinct charge states of BI are observed, namely triply, quadruply and quintuply charged. At 0.5 and 1.0 % FA, only BI$^{4+}$ and BI$^{5+}$ are observed (Fig. 2.3). However, signal intensity decreased strongly with 1.0 % FA in the mobile phase while, at 0.5 % FA, it was comparable to that obtained at 0.1 % FA. BI$^{4+}$ was most intense at low % FA when the tip was positioned close to the MS. At high % FA, the position at which BI$^{4+}$ was most
intense was further away from the MS inlet.

**Figure 2.4:** Effect of varying the flow rate on the resulting spectrum. Left: 50 nL/min. Right: 500 nL/min. A 6-µM solution of BI in H2O:ACN:FA (90:10:0.1) was infused through a 15-µm-ID uncoated nanospray tip positioned axially with the MS heated capillary. The flow was generated using a 250-µL Hamilton syringe and the syringe pump of the LCQ MS.

The shift of the charge envelop towards lower m/z likely resulted from the higher proton concentration in the solution. As already observed, the use of more acidic conditions led to an increase in the average charge for a range of peptides and proteins [13]. Interestingly though, no BI$^{6+}$ was observed, even when the sample solution was prepared in 1% FA, whereas it was visible when applying a lower organic modifier concentration (10% ACN) at only 0.1% FA (Fig. 2.4). The charge distribution over a smaller number of ions and the large signal intensity at 0.5% FA might be an advantage for sensitive monitoring of the elution of BI. The decrease in signal intensity observed at 1%FA is likely to be due to ion-pairing effects between BI and FA or to an increase in the surface tension of the mobile phase as has been suggested for trifluoroacetic acid [18].
2.3. Results & Discussion

However, the opposite has been reported as well [19]. From a practical point of view, cases where an increase in FA concentration would be necessary are limited as a result of problems in obtaining a stable spray. Whereas the nanospray tip can be positioned rather freely at low FA concentrations, a stable spray at a high FA concentration could only be obtained at a few coordinates. Therefore, positioning the spray tip and obtaining a stable spray at high FA concentrations requires much care and attention, and is not recommended.

2.3.4 Impact of the flow rate

Eluent flow rate was shown to influence the stability of the spray [9] and its morphology [10]. Moreover, the average charge state of the analytes also varies as a result of changing the flow rate: the lower the flow rate, the higher the charge state [13]. Therefore, the influence of varying the flow rate on spectral intensity, as well as on the shape of the charge envelop for BI, was assessed using an 8- and a 15-µm-ID uncoated nanoESI tip. Tips were installed on the nanointerface at a 20° angle with respect to the heated capillary while infusing a solution of 6M BI in H2O:ACN:FA (90:10:0.1). Flow rates were varied between 50 and 300 nL/min for the 8-µm-ID nanoESI emitter and between 50 and 500 nL/min for the 15-µm-ID tip.

Larger flow rates required larger spray voltages (1.7-2.4 kV for the 15-µm-ID tip) to obtain a stable spray, as has previously been reported [12]. The 15-µm-ID tip was most sensitive at 100 nL/min. Signal intensity was a little lower though very stable at higher flow rates. The smaller-ID tip exhibited maximum performances at 50 nL/min and performed similarly to the 15-µm-ID tip at higher flow rates. The flow rates studied were not sufficiently high to see a clear advantage in using larger-ID nanoESI tips. As has already been reported [13], the charge envelop shifted toward lower m/z at lower flow rates. The ratio of intensities between BI$^6+$ and BI$^3+$ on one hand and between BI$^5+$ and BI$^4+$ varied respectively from 0.5 to 1 and from 1 to 2 when the flow was lowered from 500 down to 50 nL/min (Fig2.4). Using tips with a smaller ID (i.e. 5 µm) resulted in a greater shift in the envelop toward high charges and more intense MS signal as a consequence of the greater electric field strength and the resulting more efficient protonation of the analytes. Therefore, 5-µm nanoESI tips were used in the experiments dealing with coupling a 50-µm-ID packed column with MS (cf. Chapter 5).
2.3.5 Bare-silica, distal-coated & fully-coated nanotips

Nanospray emitters are generally uncoated or gold-coated. The simple metal-liquid junction used in conjunction with uncoated nanoESI tips is expected to conduct current efficiently from the point where the potential is applied up to the opening of the nanotip. Unfortunately though, there is a voltage drop along the tip, due to eluent resistance. This voltage drop justified the development of coated needles. However, gold does not adhere well to fused-silica capillaries and tends to peel off quickly. An alternative was developed by New Objective, based on a proprietary multi-layer platinum coating. The coating was either applied at the distal end of the nanotip or on the complete emitter. Bare-silica emitters were compared to coated nanoESI tips. The influence of every type of coating on the spray voltage required to obtain a stable spray and on the spectra was assessed using an 8-µm-ID nanoESI tip at 200 nL/min. Tips were installed on the nanointerface at a 20° angle with respect to the heated capillary while infusing a solution of 600 nM BI in H2O:ACN:FA (70:30:0.1) at a flow rate of 20 nL/min. A much lower spray voltage was required to obtain a stable spray with fully-coated tips than with bare-silica tips. Optimal spray voltages for distal-coated tips are about half those required with the bare-silica tips (0.9-1.4 kV and 0.9-2.8kV respectively, depending on the distance at which the nanoESI tip is positioned).

Only very low voltages are required to obtain a stable Taylor cone when the nanospray tip is positioned at or very close to the MS inlet for both distal-coated and bare-silica tips. The fully-coated tips, however, are very susceptible to arcing because the electric potential is applied at the very end of the nanospray emitter. Therefore, no measurements were performed with these tips at less than 1 mm from the heated capillary.

Signal intensities are much larger (>10-times) for coated tips than for bare-silica tips (Fig. 2.5). Uncoated needles gave slightly more intense signals when the tip was positioned at about 3 mm from the heated capillary. In contrast, coated tips perform better when positioned close to the heated capillary (optimum at 1mm). The larger intensities found for coated tips are not accompanied by larger S/Ns. S/Ns are in fact larger for bare-silica than for coated nanospray tips. No hypothesis can be made as to why this is. However, it is to be noted in this context that coated needles require a longer time than bare-silica needles for Taylor cone stabilisation. This period is even longer in the case of fully-coated than distal-coated needles.

Even though fully-coated tips are fragile and require a relatively long equilibration time, they were applied for all further experiments. The more intense protein
signal provided by fully-coated tips should translate into an improvement in sensitivity in the time domain. The tip was always positioned on-axis and at 1 mm from the heated capillary.

![Graph showing signal intensity vs. distance for uncoated, distal-coated, and fully-coated tips.](image)

**Figure 2.5:** Influence of the type of nanospray tip on the intensity of the BI$^{4+}$ ion signal; bare silica (diamond), distal-coated (square) and fully-coated (triangle). A 600 nM solution of BI in H2O:ACN:FA (70:30:0.1) was infused through the 8-$\mu$m-ID nanospray tip at a flow rate of 200 nL/min using a 250-$\mu$L Hamilton syringe and the syringe pump of the LCQ MS. The tip was positioned under a 20° angle with the MS heated capillary.

### 2.3.6 Coupling nanoLC to MS

Different means of connecting a transfer capillary or a liquid chromatography column to a nanoESI emitter were investigated (Figure 2.1). Electrical contact was made by applying the high voltage on the outside of a fully-coated nanoESI tip (cf. scheme D, Figure 2.1) or directly to the liquid to be sprayed (cf. schemes A, B & C, Figure 2.1). The main difference between the set-ups lies in their ease of use and, most important, in their respective dead volumes. Most experiments described until now have been performed using the set-up shown in schemes A and B (Figure 2.1). Positioning the nanoESI tip inside the stainless steel connec-
tion without additional void volumes proved to be a difficult task. However, dead volumes remained significant. Moreover, schemes A and B require the use of relatively long nanoESI tips (≈ 4.0-5.0 cm), which contributed to post-column dead volumes. This resulted in very broad chromatographic peaks when the interface (scheme A in Figure 2.1) was used in conjunction with a 50-µm-ID column. Set-up C (Figure 2.1) was investigated because the internal geometry of the PEEK microcross is well-characterised and allows the easy and reproducible positioning of emitters while limiting post-column dead volumes. The bore of the microcross was smaller than the external diameter of both the column and the emitter. Their respective ends could thus be positioned against the inner walls of the microcross. The dead volume of set-up C depended on the PEEK connection. A PEEK microcross introduced 38 nL dead volumes. The nano-ESI tips required for set-up C were still relatively long (≈ 2.5-3.0 cm) and chromatographic peaks remained broad when coupled with a 50µm-ID packed column. However, such a set-up can probably be successfully used with packed columns with larger IDs or monolithic columns that accommodate larger flow rates, in which case dead volumes of the magnitude observed here are of less importance. This set-up presents the advantage of ease of use and modularity. Using the microcross, a make-up liquid can be added when a nanoLC separation requires non-volatile buffers to be used. Additionally, the microcross allowed infusion of a standard solution for tuning of the MS parameters.

Only set-up D (Fig. 2.1) allows the reduction of post-column dead volumes sufficiently to retain the separation efficiency achieved with 50-µm-ID columns. This set-up is built according to the set-up developed by Meiring et al. [20], in which the nanoLC column and the nanoESI tip are butt-connected. The Teflon sleeve used in this set-up allows visual inspection of possible dead volumes introduced in the system. However, this set-up requires fully-coated needles that are more fragile and sensitive to arcing [2, 7]. Nevertheless, set-up D proved to be useful for connecting packed and monolithic columns to the MS with satisfactory performance under most flow regimes. At high flow rates (≥1.0 µL/min), the spray became rather unstable [21](cf. Chapter 4). To accommodate for these higher flow rates, a commercially available interface, built for µL/min flow rates and allowing for a nebuliser gas, was modified according to the butt connection principle of scheme D [22] (cf. Chapter 6).
2.4 Conclusion

Complete characterisation of a nanoESI interface is necessary in order to perform accurate, precise, rapid and reproducible quantitative analysis. A nanoESI interface was built in-house and the different parameters likely to influence the quality of the spray and the spectra were investigated. Positioning the nanospray tip at a 20° angle with respect to the heated capillary led to better S/Ns, probably as a result of the reduced introduction of neutral species into the MS. However, signal intensity was maximum when the interface was positioned axially and close to the heated capillary. Larger % ACN in the mobile phase required lower spray voltages to obtain a stable spray, due to the lower surface tension and the facilitated evaporation of the mobile phase. At low % ACN (i.e. 10%), the spray voltage must be carefully adjusted in order to obtain a stable signal, whereas a much wider spray voltage range will give a stable spray at high %ACN. High %ACN resulted in a narrower distribution of the charge envelop. To date we have no explanation for this observation. Varying the FA concentration had a clear impact on both the intensity and the charge envelop. Larger %FA made the charge envelop shift toward lower m/z. Too high a %FA, though, led to a strong reduction in signal intensity. This may have been due to an ion-pairing effect between the peptide and formate ions, or to the increased surface tension of the mobile phase. Larger intensities were found for coated needles. Unexpectedly, S/Ns were higher for bare-silica than for coated-nanospray emitters. Lower flow rates required lower spray voltage and resulted in higher charge states of the analyte. Using tips with smaller ID resulted in an even greater shift of the charge envelop toward high charges. In addition a more intense MS signal is obtained as a consequence of the greater electric field strength, leading to more efficient protonation of the analytes.
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