The effect of ultra-low flow on the ionization of biotherapeutics

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ABSTRACT
Biotech drugs, especially monoclonal antibodies have emerged as very promising new generation protein drugs. However, most preparation and analysis methods are used at high flow. These single droplet modes require strict control but their inherent complexity makes it hard to control most factors in not easily achievable. This approach is the creation of capillary electrophorograms with electrospray ionization is a single dynamic process (CIESI) coupled with high-resolution mass spectrometry (HRMS) to pave the way to this end and at every level of the characterization of complex mixtures. The application of low flow rates in the analysis of biotargets suffers from the ion suppression phenomenon, which is systematically investigated in this presentation. Some of the main advantages of CIESI is the ability to produce stable electrophoretic ultra-low flow rates (0.05 nL/min range) in a robust and reliable manner. In this presentation, the effect of flow rate on sensitivity, ion suppression and detection sensitivity will be discussed. Our latest research project analyzed biological samples by the ultra-low flow (at 20 nL/min to 3 nL/min) sample introduction which can be used in compaction of biological systems.

SALARY PREPARATION
Mixtures of maltotetraose (M = 644.49 g/mol) and maltotetraose (M = 644.49 g/mol) were prepared in equivalent concentrations of intact oligosaccharides (at 3 nL/min, in a mixture of 10 mM ammonium acetate buffer solution and methanol) for the intact protein analysis was explored at a concentration of 5 nM in the form of nL.

INSTRUMENTATION
Flow rates of 3 nL/min to 250 nL/min were carried out with online hybridization of a CIESI (Biologic C1000, Biologic, Inc., Sligo, Ireland) and 120 nL sample size. (Thermo Scientific Exactive, Bremen, Germany). The ionization process was performed with capillary electrophoresis coupled with electrospray ionization with a 10 cm column (10 cm x 100 μm inner diameter). Details on the ionization process was performed with capillary electrophoresis coupled with electrospray ionization with a 10 cm column (10 cm x 100 μm inner diameter). Details on the ionization process were given in previous studies. The injection system was designed and realized during this study. For the intact protein analysis experiments with the following setup: capillary temperature, 30°C, 1 kHz voltage, 4500 V and 100 A laser intensity. All experiments were carried out at an injection pressure (injected proteins) that did not need to be considered.

ION SUPPRESSION
The liquid flow rate in CIESI determines the droplet size, thus plays an essential role in the efficiency of the technique. It has been reported that pressure rectification, where ion suppression is essentially low or non-existent, drop sizes in the range of 10–50 μm can be achieved by the optimization of both the liquid and gas flow rates in the nebulizer system of a CIESI 8000 instrument and were estimated determined using the Winer-Ploucquet equation:

fig.1

where D is the pressure difference, Δp is the hydraulic viscosity, D is the liquid flow rate, L is the liquid length, g is the acceleration due to gravity, R is the capillary radius, V is the liquidnel flow rate, S is the liquid surface tension, and K is the wall wetting factor. The ion suppression phenomenon is systematically studied using a well-defined oligosaccharide – maltotetraose, which is considered as weakly ionizable carbohydrate. The other hand is an easily protonated peptide. Figure 1 shows the signal intensity ratio of the increasing the liquid flow rate. As can be seen, the flow rate is 100 nL/min, with respect to the liquid flow, decreases the ion suppression phenomenon. As a first approximation we can consider the signal intensity ratio of the droplets in the ion suppression phenomenon. One can see that these droplets are significantly higher than the signal intensity ratio of the droplets in the ion suppression phenomenon.

CONCLUSIONS
Normalized signal intensities at 20 nL/min (left) and 250 nL/min (right) flow rates. Sample: 3 µM maltotetraose.

REFERENCES

Table 1. Analytical signal intensity and characterization at different flow rates.

Table 2. Analytical signal intensity and characterization at different flow rates.

Table 3. Analytical signal intensity and characterization at different flow rates.

Figure 1. Ion suppression equation.

Figure 2. Characteristic MS spectra of a 1 nM monoclonal and neotensin mixture at 9 nL/min flow rate.