# Analysis of oligonucleotides by capillary zone electrophoresis-electrospray ionization quadrupole time-of-flight mass spectrometry.

GHENT UNIVERSITY

An V. Willems[1], Dieter L. Deforce[2], Carlos H. Van Peteghem[3], and Jan F. Van Bocxlaer[1]

[1]Laboratory of Medical Biochemistry & Clinical Analysis, [2]Laboratory of Pharmaceutical Biotechnology, [3]Laboratory of Toxicology, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium
e-mail address: Anv.Willems@UGent.be

## 1. Introduction

• Synthetic oligonucleotides e.g. PCR primers, probes, antisense therapeutics ⇒ no defect in length or sequence tolerated

• Quality control and characterization of oligo's after synthesis: accurate and rapid structural identification and purity determination

• Analytical tool: electrospray ionization mass spectrometry (ESI-MS)

- molecular weight determination

- deconvolution algorithm produces zero charged spectrum from multiply charged ESI raw spectrum

- single base substitutions? (between 9 and 40 Da)

• Problem: adduction of sodium or potassium ions to polyanionic backbone

⇒ highly complex mass spectra

• Desalting possible by replacement of metal ions with ammonium ions (less tightly bound to oligo, dissociate during electrospray process) or by addition of chelating agents (e.g. *trans*-1,2-diaminocyclohexane-*N,N,N,N*-tetraacetic acid (CDTA)) or piperidine and imidazole to the spray solvent

• Objective: development of an on-line capillary zone electrophoresis (CZE)-negative nano-ESI-MS method with an ammonium carbonate buffer using a Q-TOF mass analyzer for the characterization of oligo's including concomitant removal of salt ions

## 2. Experimental

### Oligonucleotide samples:

• Samples (Applied Biosystems): Table 1 (between 125 and 180 pmol/µL)

• Oligonucleotide 3: model oligo for the development of the method

### CZE-MS conditions:

•CZE system: PRINCE (Lauerlabs), fused silica capillary (0.86m x 50µm i.d.)

• Mass spectrometer: Q-TOF (Waters Corporation, Manchester, UK)

• Ion source: triaxial nano-electrospray source (Z-spray®) in the negative ion mode

• Hydrodynamic injection (100 mbar, 1 min) followed by preconcentration on the capillary using sample stacking

• Electrophoresis buffer: 25 mM ammonium carbonate (pH 9.7) (+ 2.5 mM piperidine and imidazole/+ 0.2 mM CDTA/+ 2.5 mM piperidine and imidazole + 0.2 mM CDTA)

• Electrophoretic conditions: 14 kV, 60 mbar

• (-)-ESI conditions: capillary voltage –3.0 kV, cone voltage 35 V, source temperature 80°C, desolvation gas flow rate 125 L/h and nebulization gas pressure 1.2 bar.

• Sheath liquid: 80/15/5 isopropanol/water/0-20 mM ammmonium carbonate pH 9.7 (0.7 µl/min).

## 3. Results and discussion

### Goal

• Optimization of parameters to obtain stable electrospray conditions, the best signal response for the oligonucleotides and a maximum reduction of the nonvolatile cations while maintaining a reasonable analysis time

• Results

- Bar diagram: sum of the signal abundances (peak heights) of the different multiply charged ions of oligonucleotide 3 and of all the observed adducts, extracted from the full scan spectra of the sample

- Line diagram: ratio of the sum of the peak heights of the different multiply charged ions of oligonucleotide 3 and the sum of the peak heights of all the observed adducts (the higher the ratio, the better the desalting occurred)

### Preliminary experiments

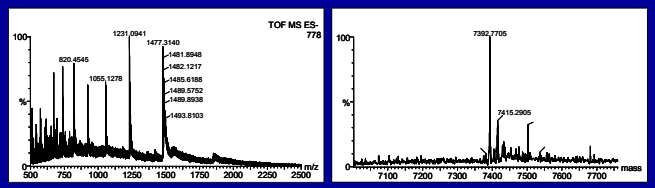
• Buffer: 25 mM ammonium carbonate (metal ions are exchanged for ammonium ions during separation)

• Selection of electrophoretic conditions: 14 kV, 60 mbar

⇒ stable electrospray, 25 min analysis time

• pH optimization in range from 7.0 to 10.3 ⇒ pH 9.7 best results

• BUT: still sodium and potassium adducts visible in (deconvoluted) spectrum (Figures 1 and 2)

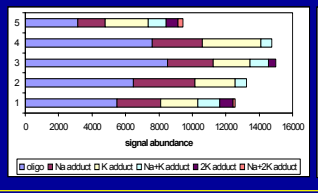



Figures 1 and 2. Spectrum (left) and deconvoluted spectrum (right) of oligonucleotide 3 using 25 mM ammonium carbonate (pH 9.7) as electrophoresis buffer.

### Optimization of electrophoresis buffer concentration and composition

• Tested: 25 mM ammonium carbonate (1), 50 mM ammonium carbonate (2), 25 mM ammonium carbonate + 2.5 mM piperidine and imidazole (3), 25 mM ammonium carbonate + 0.2 mM CDTA (4) or 25 mM ammonium carbonate + 2.5 mM piperidine and imidazole + 0.2 mM CDTA (5) ⇒ Figures 3 and 4

• 25 mM ammonium carbonate + 0.2 mM CDTA: highest increase of signal response of oligonucleotide, best reduction of cation adducts



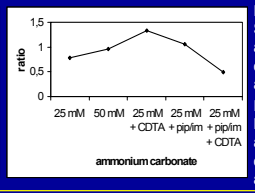




Figure 3 (left): Signal abundance of oligo 3 and adducts.
Figure 4 (right): Ratio of signal abundance of oligo 3 and adducts.

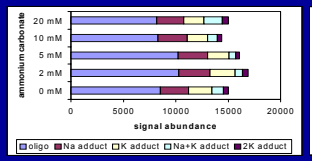
### Optimization of the sheath liquid composition

• Isopropanol better as organic solvent than methanol or acetonitrile

• 50, 60, 70 or 80% isopropanol ⇒ selected: 80% isopropanol

• addition of 5% 0, 2, 5, 10 or 20 mM ammonium carbonate (pH 9.7)

⇒ selected: addition of 5% 5 mM ammonium carbonate (Fig. 5 and 6)



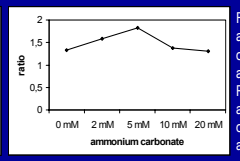




Figure 5: Signal abundance of oligo 3 and adducts. Figure 6: Ratio of signal abundance of oligo 3 and adducts.

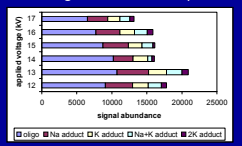
### Optimization of the CZE voltage

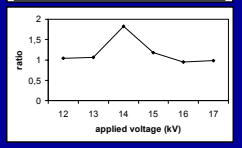
•Tested: 12, 13, 14, 15, 16 or 17 kV ⇒ 14 kV selected (Figures 7, 8 and 9)

### Application

• 7 other oligonucleotides (Table 1, average of 3 measurements + standard deviation), little adducts observed in spectra (example Figure 10)

• Maximum errors < 55 ppm or 0.3 Da ⇒ smallest difference (A to T switch differing 9 Da in mass) can be detected

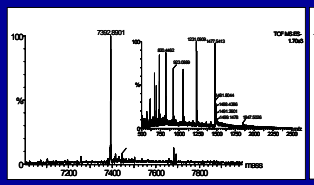


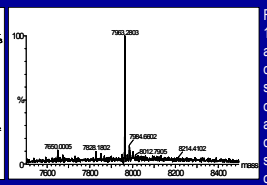




| No | Base composition | Expected MM | Observed MM (SD) | ppm |
|---|---|---|---|---|
| 1 | 5'-CCA CCA TGC CAC CTC CT-3' | 5011.3092 | 5011.0369 (0.0928) | -54.3 |
| 2 | 5'-GGT GCT CCA GGT GCC CAT-3' | 5491.6027 | 5491.6204 (0.1841) | 3.2 |
| 3 | 5'-CCC TGG GCT CTG TAA AGA ATA GTG-3' | 7392.8587 | 7392.8936 (0.0152) | 4.7 |
| 4 | 5'-ATC AGA GCT TAA ACT GGG AAG CTG-3' | 7425.8973 | 7425.6104 (0.0953) | -38.6 |
| 5 | 5'-AAT AAG CTT CCA CCA TGC CAC CTC CT-3' | 7795.1307 | 7795.1129 (0.1392) | -2.3 |
| 6 | 5'- ATT GTC GAC GGT GCT CCA GGT GCC CA-3' | 7963.2024 | 7963.3169 (0.1779) | 14.4 |
| 7 | 5'- ATT GTC GAC GCT CTT CAT CCT CGT TCT CA-3' | 8745.7164 | 8745.8452 (0.1485) | 14.7 |
| 8 | 5'-ATT GTC GAC CAC AGC TGA GAC CTT CCA GCC-3' | 9111.9673 | 9111.9255 (0.1282) | -4.6 |

Tabel 1: Oligonucleotides used in this study.

Figure 7 (left up): Signal abundance of oligo 3 and adducts. Figure 8 (left down): Ratio of signal abundance of oligo 3 and adducts.




Figures 9 and 10: Spectrum and deconvoluted spectrum of oligo 3 (left) and deconvoluted spectrum of oligo 6 (right).

## 4. Conclusion

It is concluded that the CZE-ESI-MS method with on-line sample stacking can remove salt ions of oligonucleotide samples, deleterious for mass spectrometric oligonucleotide length and sequence analysis. The procedure uses minimal labor and little sample, thus it is ideally suited for the quality control of oligonucleotides.

### 5. Acknowledgements

We wish to thank Ing. S. Vande Casteele for all her help with the analysis of the samples. This work was supported by grant GOA99-120501-99 (Eigen OnderZoeksFonds).