A case study of (TAGGGT)2 G-quadruplex oligonucleotides by non-equilibrium kinetics capillary electrophoresis coupled to native ion mobility mass spectrometry

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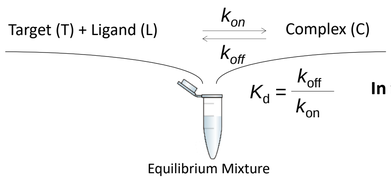
Introduction

Oligonucleotides are notably known to form G-quadruplex structures in the presence of various cations, including ammonium, sodium or potassium ions. Melting point experiments using Circular Dichroism detection, nuclear magnetic resonance, native mass spectrometry and native ion mobility mass spectrometry were extensively used to investigate the structures of these G-quadruplexes in solution and the gas phase. Nonetheless some discrepancies appear concerning the experimental conditions allowing the preservation of the G-quadruplex structures in the liquid phase and after the transfer to the gas phase.

We propose to introduce non-equilibrium capillary electrophoresis hyphenated to native ion mobility mass spectrometry to investigate the contribution of the main experimental parameters such as the concentration of coordinating cations in the background electrolyte, composition of the electrolytes and temperature, sheath liquid composition of the CE-MS interface, and sample preparation of the G-quadruplexes

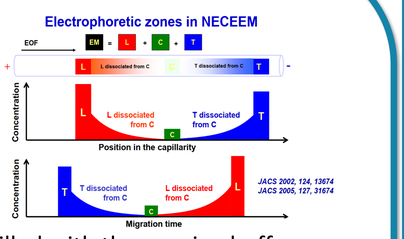
Material & methods (2)

Non-Equilibrium Capillary Electrophoresis of Equilibrium Mixtures



In the case of the G-quadruplex formation :

$2\ TAGGGTTAGGGT + 2NH_4^+ \leftrightarrows [(TAGGGTTAGGGT)_2 + 2NH_4^+]$



- A short plug of the equilibrium mixture is injected into the capillary prefilled with the running buffer
- The separation is carried out with both inlet and outlet reservoirs containing only the running buffer
- The complex (in this case, the G-quadruplex) continuously dissociates during electrophoresis
- The resulting electropherogram contains 3 peaks for T, C and L with 2 exponential « smears » of L and T resulting from the dissociation from C

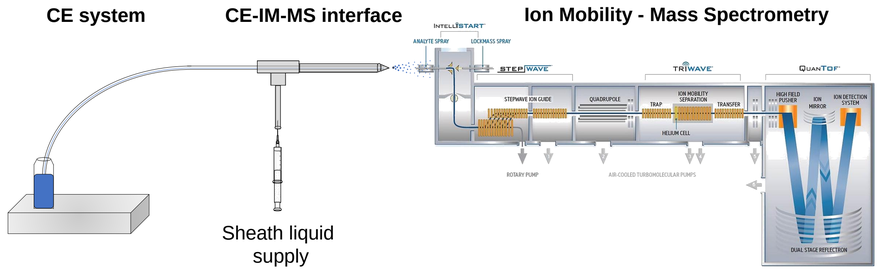
Materials & methods (1)

➢ **Sample preparation**:

NH_4^+ G-quadruplex : 50µM $(TAGGGT)_2$ in 100mM NH_4Ac, 60% ethanol (EtOH) unless otherwise stated

➢ **Methods**:

Capillary Electrophoresis (CE): CESI8000 equipped with an EDA (Sciex)
Capillary : 90cm x 365µm x 50µm. Normal polarity performed @ 30kV
CE-(IM)-MS Interface: home-made low-flow sheath liquid CE-MS interface
Ion Mobility Mass Spectrometry (IM-MS): Synapt G2 HDMS (Waters)
Negative ion mode, cone voltage at 3.0kV, IMS wave conditions: 40V - 1000m/s N_2 buffer gaz 90mL/min

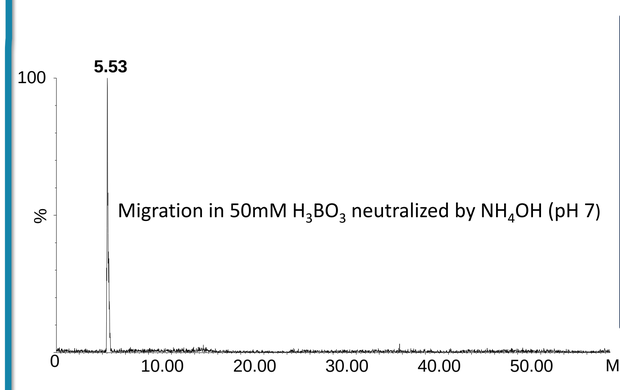


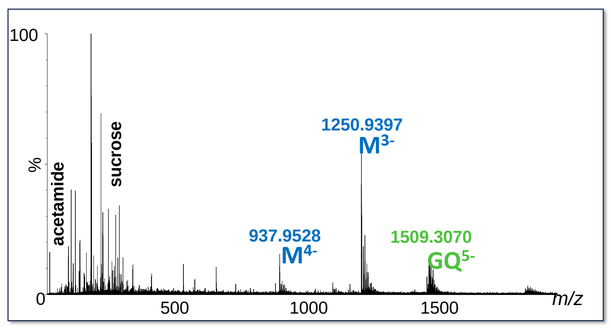
➢ **Data processing**

- Thermodynamic properties of G-quadruplexes have been computed from the parameters of an exponential function fitting the electropherogram (NECEEM). This fitting has been performed using a Python script involving the Scikit-learn and SciPy libraries directly on the exported electropherogram. RANSAC and Savitzky-Golay filter algorithms have been applied on the data to refine the exponential fit.
- CE-IM-MS data have been processed with our Mass Spectra Kendrick Filter[(2)] (MSKF v2.0) to generate arrival time distributions (IM) correlated to specific migration times (CE).

Results & Discussion

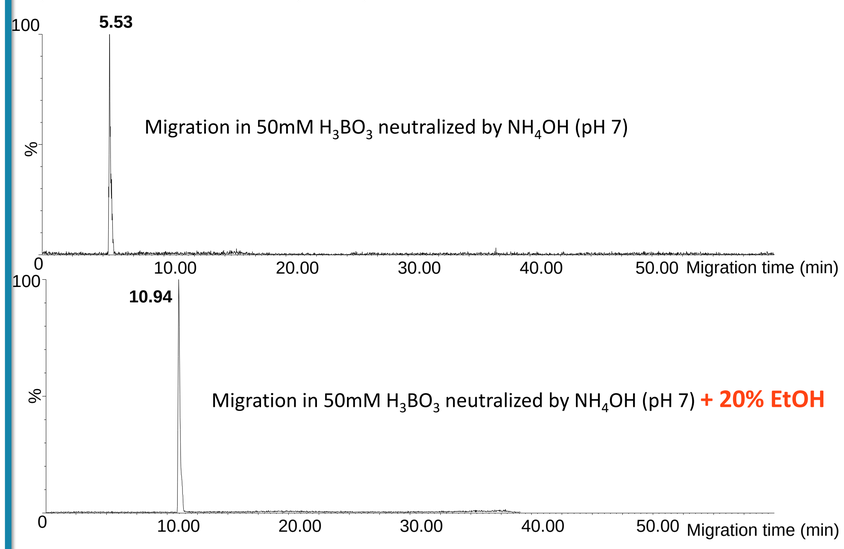
❖ **Standard run conditions for NECEEM analysis : boric acid as background electrolyte**





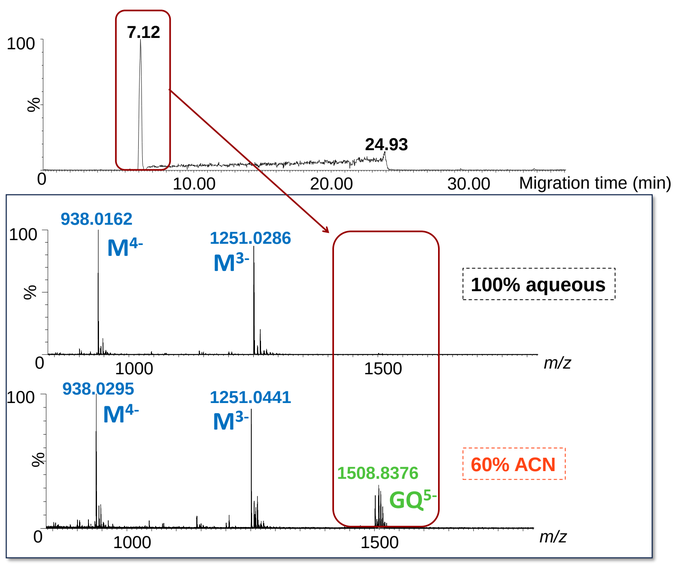
Investigation of experimental parameters

1) Presence of ethanol (EtOH) inside the BGE



- The addition of EtOH brought no supplementary information
- The higher migration time is due to a lower conductivity in the capillary caused by the addition of an organic solvent

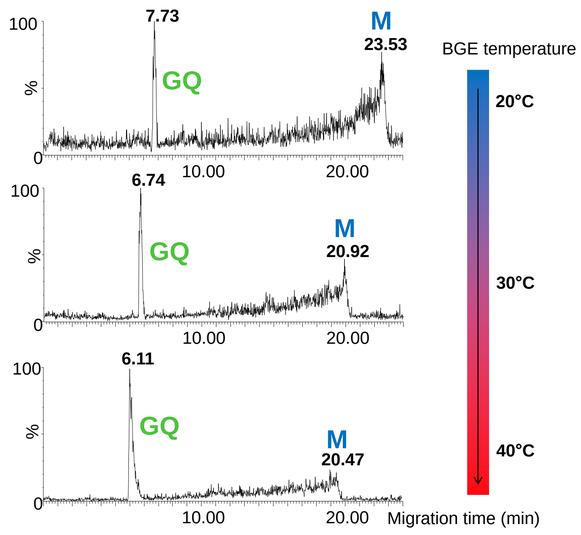
2) Sample preparation : addition of organic solvents



- Drastic effect of the addition of organic solvent in the sample preparation on the detection of the G-quadruplex

→ **Higher intensity** detected for the GQ

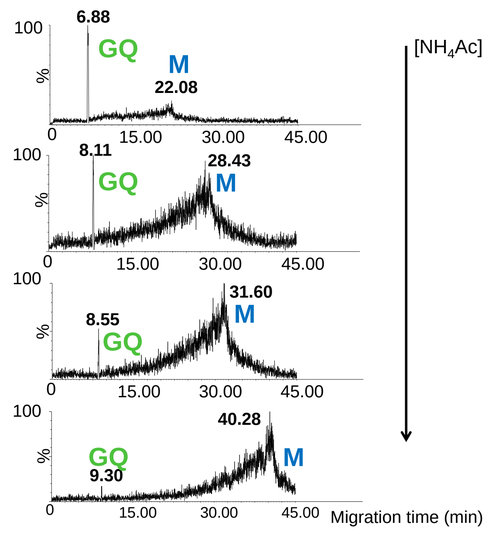
3) Increase of BGE temperature



- Noticeable effect of the BGE temperature

→Increasing the temperature of the BGE causes a lower extend of GQ dissociation

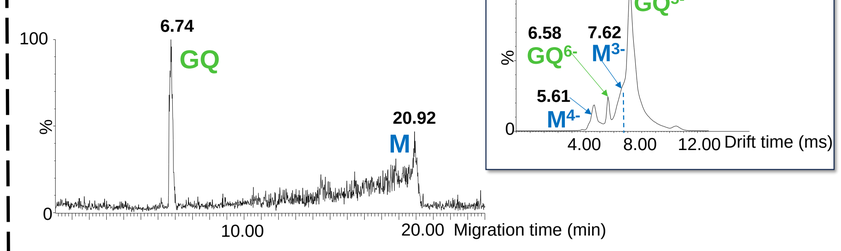
4) Increase of ammonium concentration in the BGE



- Noticeable effect of $[NH_4Ac]$ concentration

→Increasing the concentration of ammonium ions inside the BGE causes an extended tailing of GQ dissociation into its constitutive monomer (M)

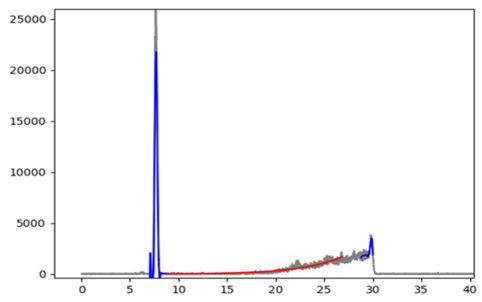
Introduction of ion mobility spectrometry (IMS) into the workflow



- Gas phase characterization of the species separated in solution by CE

Dissociation constant estimation

1. Fit of the electropherogram to obtain the apex of the GQ peak (+/-7min) and the peak related to the monomer (+/- 30min) : **blue traces**
2. Exponential fit of the region related to the dissociation of the G-quadruplex during electrophoresis (between both peaks): **red trace**



$$I_t = I_{t_{monomer}} \exp\left\{k_{off} \frac{t_{G\ quad}}{t_{monomer} - t_{G\ quad}} (t - t_{monomer})\right\}$$

The exponential fit of the data leads to a dissociation constant value of $k_{off} = 0.693 min^{-1}$

Future work based on the use of the integration of each contribution in the electropherogram will lead to the determination of K_D

Conclusion

CE(-IM)-MS investigation of G-quadruplex:

- ➢CE → information about G-quadruplex in solution (electropherogram)
- ➢IM → information about G-quadruplex in the gas phase after the ionization process (arrival time distribution or ATD)
- ➢MS → molecular formula + possible structural information (MS and/or MS/MS spectra)

Experimental parameters optimization :

- ➢MS and CE-MS interface:
  - Use of low sampling and extraction cone voltages + low T° to best preserve the G-quadruplex during ionization
  - Optimized sheath liquid based on 80% IPA + 5mM NH_4Ac + 5µM TEA for efficient negative ion mode
- ➢CE : use of boric acid based BGE (high EOF) as reference BGE
  - increasing $[NH_4^+]$ promotes G-quadruplex dissociation
  - Increasing BGE T° promotes G-quadruplex dissociation
  - Sample preparation: organic solvent dramatically affects the MS detection of the G-quadruplex after migration

Use of NECEEM :
Fitting the data in the exponential decay area leads to the determination of the dissociation constant (k_{off})

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