SIELC

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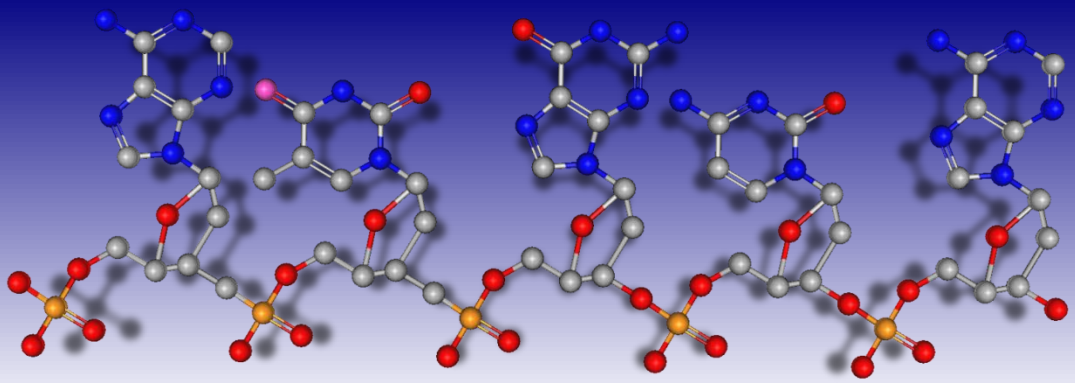
Newsletter



New Way of Separation of Oligonucleotides with only H_2O/MeCN in Mobile Phase

Many organic molecules of medical, pharmaceutical, industrial, and environmental interest can exist in an ionic form at specific pH levels. One such example is Oligonucleotides (OGNs), which are an important group of negatively charged molecules used in a variety of applications including medicine, research, and forensics. Liquid chromatography (LC) separation of this class of molecules is an important step in their production, characterization, and purification. There are several separation modes based on different properties of the oligonucleotide molecules.

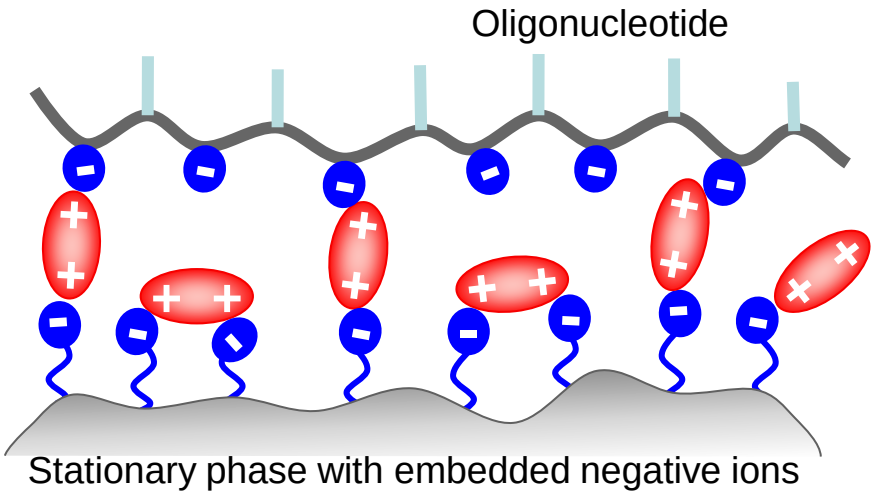


Fig. 1
Non solvated double charged positive ions forming a bridge holding oligonucleotides on the column surface.

We recently reported on a new mode of liquid chromatography which we named Bridge Ion Separation Technique (BIST ®). Oligonucleotides are a type of molecules that can be efficiently separated by this new technique.
The method requires some amount of organic modifier in the mobile phase and a double-charged positive ion to be dynamically loaded on a cation exchange stationary phase (Fig. 1) to create the bridge formation between negatively charged stationary phase and negatively charged oligonucleotides. We found that the best result was obtained when Mg^{2+} was used as double-charged positive ions for the bridge formation.

An important property of BIST® mode separation is the amount of water in the MP, which affects the level of solvation of the double-charged ions and correspondingly the degree of retention of oligonucleotides. By reducing the amount of the organic modifier, any oligonucleotide can be eluted from the column Fig. 2, 3.

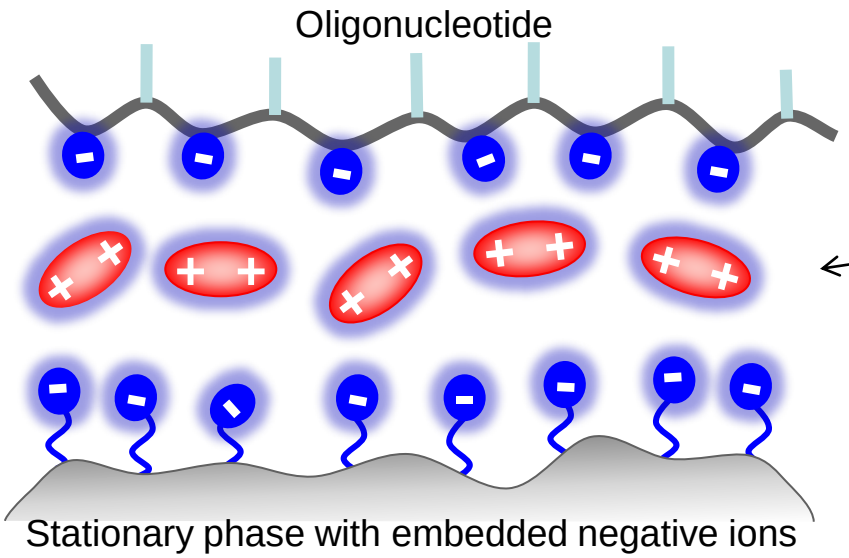


Fig. 2 Solvation of double charged ions affects degree of bridge formation and degree of retention of oligonucleotides

It turns out that the double-charged ions do not need to be supplied continuously by the MP. Once loaded with such ions the column can be operated with only H_2O/MeCN mobile phase for many hundreds of injections Fig. 4. Because of the buffer free (ion-free) mobile phase, the bridging double-charged ions have very low mobility and can not be easily removed from the stationary phase, however they still provide bridge formation for the many repetitive analyses.

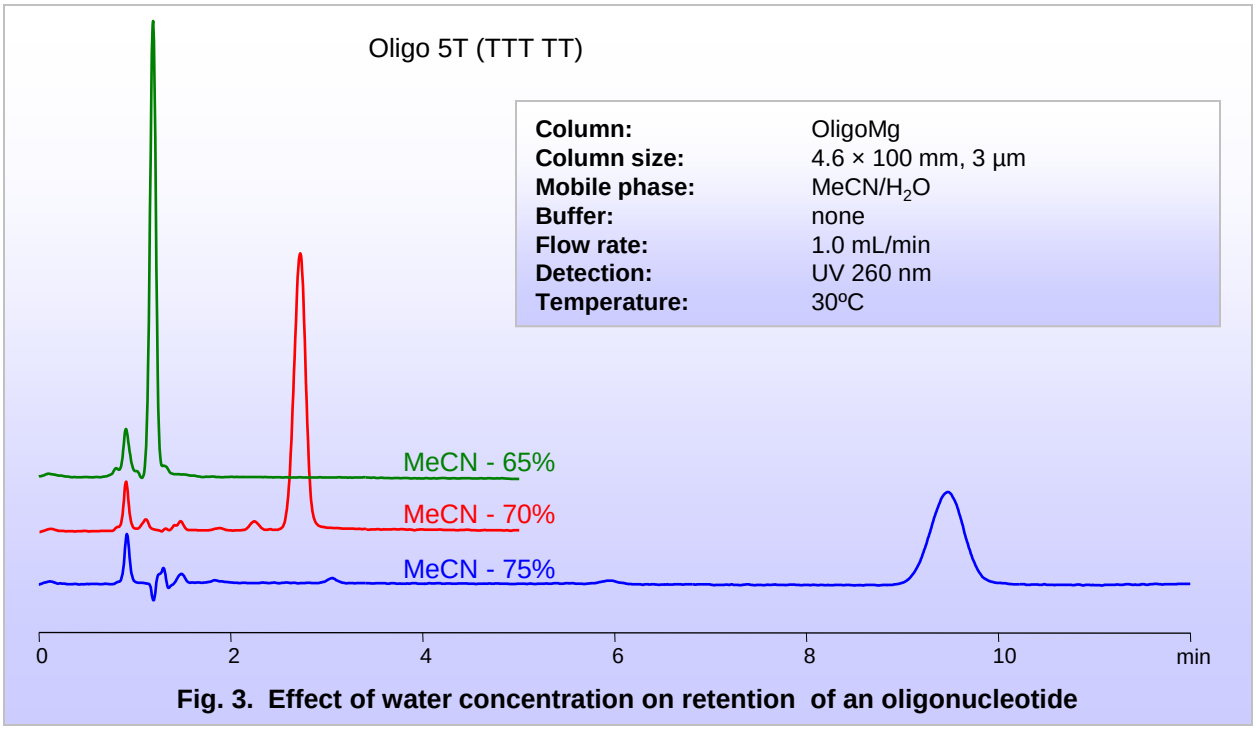


Fig. 3. Effect of water concentration on retention of an oligonucleotide

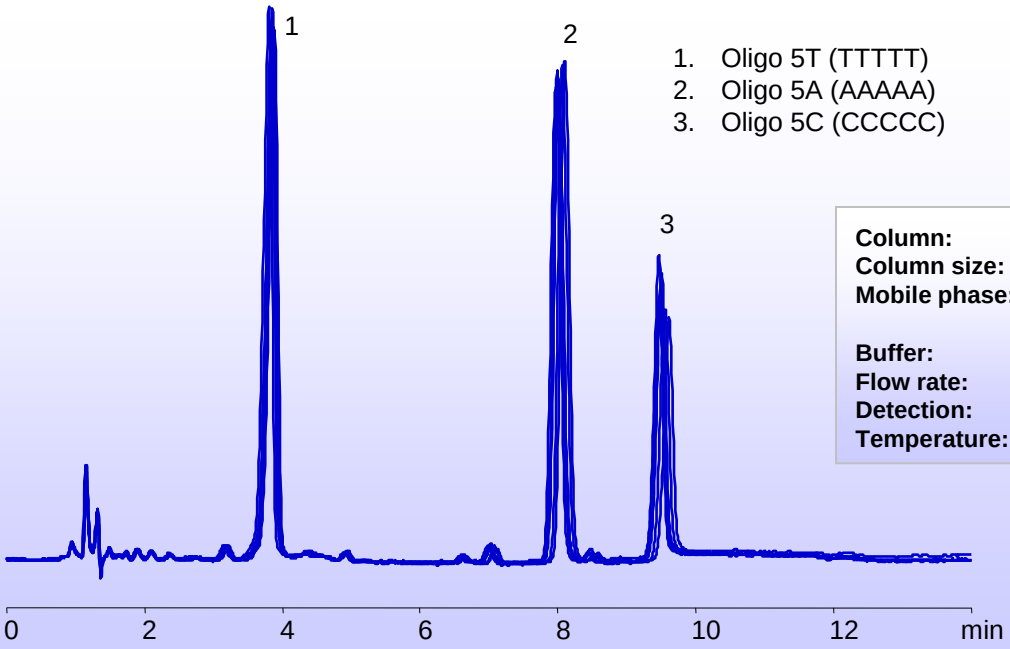


Fig. 4. 50 consecutive injections of three oligonucleotides mixture

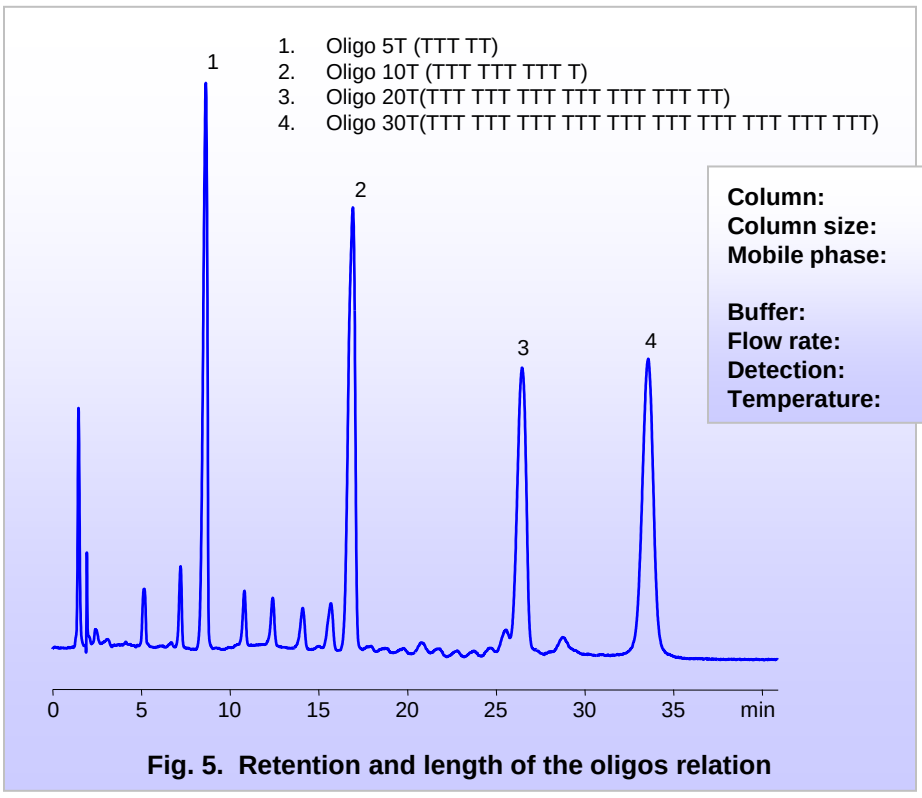
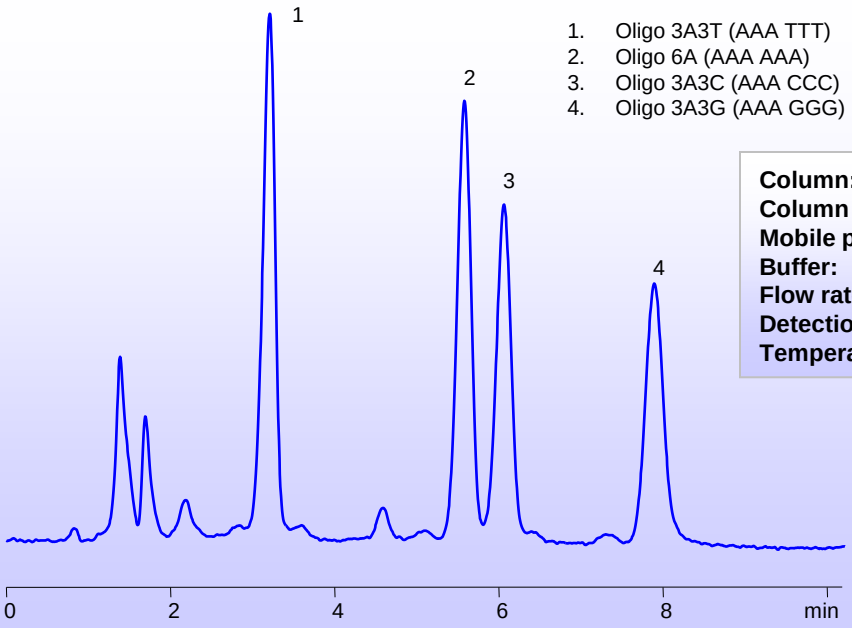


Fig. 5. Retention and length of the oligos relation

The retention of oligonucleotides in general correlates with the number of nucleobases. This is in agreement with bridge formation phenomena, since the longer chain of the molecule will have more negative charges available for bridging and longer retention will be observed. Another aspect that affects retention and the bridge formation is the geometry of the oligo-molecules. This is clearly demonstrated that the same length sequences provide very different retentions when their nucleobases are different Fig. 4, 6.

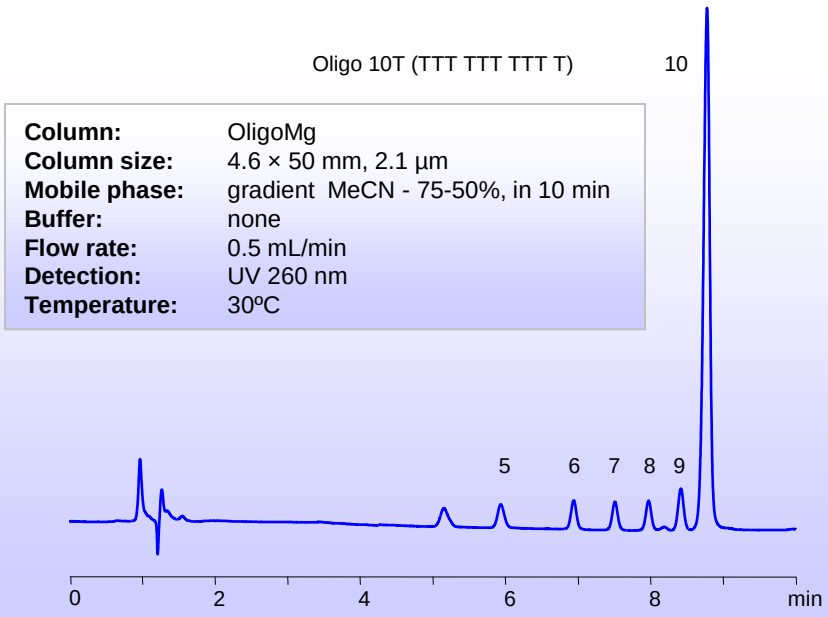


Column:	OligoMg
Column size:	4.6 × 150 mm, 3 µm
Mobile phase:	gradient MeCN - 65-45%, in 10 min
Buffer:	none
Flow rate:	1.0 mL/min
Detection:	UV 260 nm
Temperature:	30ºC

Fig. 6. Retention difference of 6 mer oligonucleotides

For BIST® to work three conditions need to be met:

- *Cation exchange column with embedded negatively charge functional groups*
- *A double-charged positive ions should be loaded on the column*
- *Reduced water in the mobile phase to control the level of solvation of the bridging ions*



Column:	OligoMg
Column size:	4.6 × 50 mm, 2.1 µm
Mobile phase:	gradient MeCN - 75-50%, in 10 min
Buffer:	none
Flow rate:	0.5 mL/min
Detection:	UV 260 nm
Temperature:	30ºC

Fig. 7. Retention of 10T oligo and its shorter byproducts

Summary

One important advantage of this BIST® separation if compared to an ion-exchange mode is complete removal of the buffer from the mobile phase. When compared to a Reverse-Phase Ion-Pairing separation of oligonucleotides, the advantages are a simple MP composition, quick equilibration time with gradient mode and no interference with other molecules retained by RP mechanism. BIST® also offers different selectivity when compared with any other separation mode.

Every new separation mode offers new possibilities in complex analytical or prep situations.

OligoMg columns available in many standard sizes with particles 2, 3, and 5 µm and pores size 110 Å.