

# Prediction of oligodeoxyribonucleotides eluting salt conditions on anion exchange column

J.L.Vaerman, P.Moureau, Y.Larondelle, P.Martiat and M.Philippe

Laboratoire de Biologie Moléculaire Clinique, Cliniques St Luc, Université Catholique de Louvain, Clos Chapelle aux Champs 30/3046, 1200 Bruxelles, Belgium

Submitted May 21, 1992

Chromatography is commonly used as a purification method for synthetic oligodeoxyribonucleotides (1). Anion exchange chromatography on Mono Q column (Pharmacia), under denaturing conditions and using non-organic buffers, gives adequate purity for sequences up to 50 bases long (2). The eluting NaCl concentration primarily depends on the oligonucleotide length. However, base composition and sequence also influence these eluting concentrations (Figure 1).

We elaborated a simple experimental law which predicts eluting NaCl concentrations for oligonucleotides, on the basis of their base composition and length. The equation was established for oligos from 10 to 50 bases long chromatographed under the following conditions: column Mono Q HR 5/5 (Pharmacia); elution with a freshly prepared solution of 10 mM NaOH (pH 12.0) and a salt gradient from 0 or 0.5 to 1.0 M NaCl (5 mM NaCl/min); flow = 0.5 ml/min; sample load = 200 micrograms of crude synthetic oligonucleotide. Homopolymeric oligonucleotides nA, nC, nG, nT were synthesized (Phosphoramidite chemistry on Pharmacia Gene Assembler Plus, n = 5; 10; 15; 20) and subjected to chromatography. The correlation equation between oligonucleotide length logarithm and eluting salt concentration was calculated for each homopolymeric family (Figure 2). The predictive law was constructed on the basis of these four previous equations:

$$\text{NaCl}(\text{theo}) = ((-15 + 42\log N)A + (-14 + 45\log N)C + (34 + 40\log N)G + (29 + 33\log N)T)/(100N)$$

In this relation, NaCl(theo) represents the predicted salt concentration (mol/l) needed for the elution of the oligonucleotide. N is the length of this oligomer and A, C, G, T represent the number of times each base appears in the sequence.

To test the validity of the proposed formula, we plotted the predicted (NaCl(theo)) versus experimental (NaCl(exp)) elution conditions for 71 different oligonucleotides (Figure 3). The calculated error estimation in the predicted values is 50 mM NaCl (linear correlation, error = 2 sigma) for oligonucleotides length ranging from 12 to 45 bases.

This predictive law not only illustrates the base-specific interactions between Mono Q column (Pharmacia) and oligonucleotides but also permits rapid oligonucleotide purification and analysis optimisation.

## REFERENCES

1. Gait M.J., Matthes H.W.D., Singh M., Sproat S. and Titmas R.C. (1982) *Nucleic Acids Res.* **10**, 6243–6253.
2. Deijl C.M. (1986) *Proceedings Second FPLC Symposium*, Pharmacia Biotechnology, 57–64.

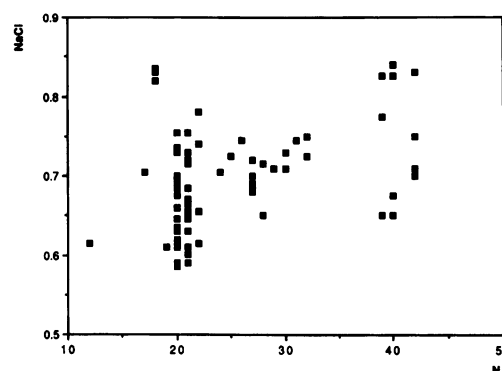


Figure 1. Relation between oligonucleotide length (N) and eluting conditions (NaCl, Mol/l) for 71 different oligonucleotides.

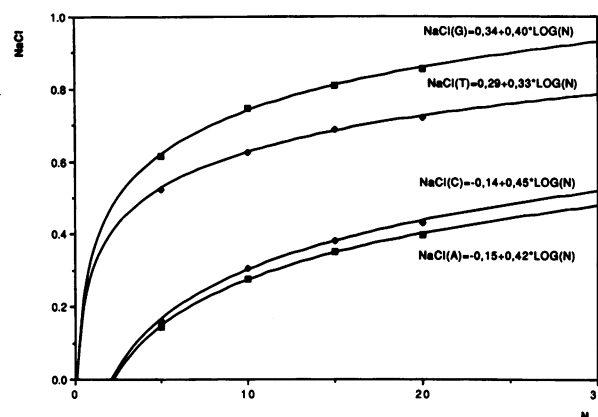


Figure 2. Correlation between homopolymeric oligonucleotides length (N) and eluting conditions (NaCl, mol/l).

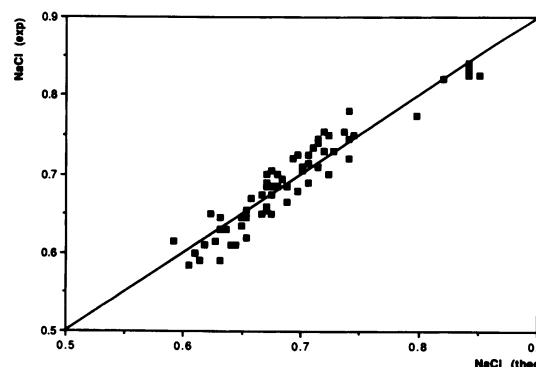


Figure 3. Predicted (NaCl(Theo), mol/l) versus experimental (NaCl(exp), mol/l) eluting conditions for 71 different oligonucleotides.