Collection Method of Aptamers

Application note Ref : AN 2.006-V1

Aptamers are DNA (or RNA) oligonucleotides selected from random libraries of DNA sequences. They fold into a three dimensional structure to bind different classes of targets with high affinity and selectivity. Both the chances for the aptamer to be selected and the quality of the selected aptamer are largely dependent on the method selection.
In this note, we optimised the parameters of collection on a standard mixture to validate the collection method in a small range of migration times to get the slower koff.

Capillary Electrophoresis Collection Method

In the aim to standardise a collection method, we analysed a mixture of three sugars: glucose (DP1), maltose (DP2) and maltotriose (DP3) labelled with APTS. We injected a plug of 100nL at $c_i=10^{-7}$ mol/L and we measured each migration time.
Then, we set up a collection method and we performed 8 collections. Sugars were collected separately in three vials containing 15µL of buffer (Fig.1).The collected samples were injected again to estimate the yield and the reproducibility of our collections (Fig. 2).

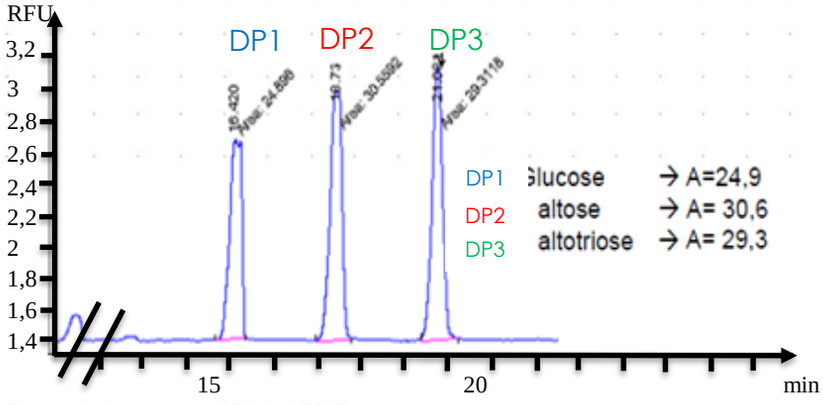


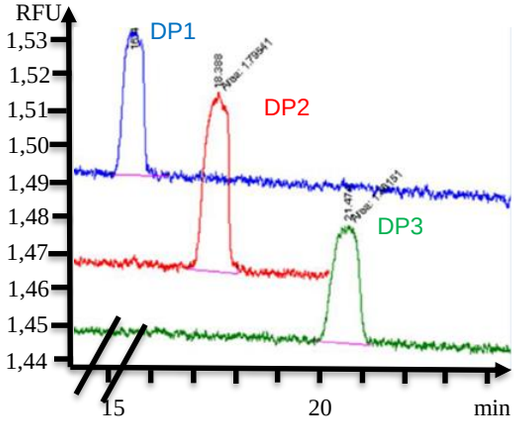
***Figure 1**: Analysis by CE-UV-*LEDIF *of three sugars (tm: migration time; A:area)*
DP1 t_m=16.4min – A1=24.9; DP2 t_m=18.7min – A2= 30.6; DP3 t_m=21.1min – A3= 29.3

Instruments:
Capillary Electrophoresis: Agilent Technologies CE7100
Detector: Picometrics ZETALIF LED 480nm/30nm

Sample:
Glucose (DP1), maltose (DP2) and maltotriose (DP3) are labelled with APTS and diluted in water.
A plug of 100nL is injected into the capillary.

Method:
- Capillary: 90cm x 50µm ID (effective length 69cm)
- Migration buffer: 40mM aminocaproic acid pH=4.5 + 0,02% hydroxypropylmethylcellulose.
- Voltage: +20kV
- Injection: 30mbars, 10s
- Cassette temperature : 25°C

We checked if the peak area of the collected products corresponds to the dilution factor DF:
$c_i=10^{-7}$ mol/L; v_i =100nL 8 collects and v_f =15µL so DF = $8*10^{-7}$ /$15*10^{-6}$ = 0.05.



DP1 Glucose → A=1.2
DP2 Maltose → A= 1.8
DP3 Maltotriose → A=1.4

$AF1_{theorical}$=DF*AI1= 24.9*0,05=**1.3**	⇔	$A1_{experimental}$= **1.2**
$AF2_{theorical}$=DF*AI2= 30.6*0,05=**1.5**	⇔	$A2_{experimental}$= **1.8**
$AF3_{theorical}$=DF*AI3= 29.3*0,05=**1.5**	⇔	$A3_{experimental}$= **1.4**

Migration times are different due to the fact that samples were collected in a buffer.

***Figure 2**: Injection of products collected by CE-UV-LEDIF DP1 t_m=16.4min – A1=1.2 ; DP2 t_m=18.4min – A2=1.8 ; DP3 t_m= 21.5min – A3=1.4*

Conclusion:

We are able to collect each of three sugars with a yield close to 100%.
We developed an efficient method of complex separation which leads us to collect aptamers with an excellent yield and to overpass these bottleneck steps.