Combining Electrophoretic Mobility with AF4 separation for Modified Pectin

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SEC/FFF-MALS • CG-MALS • DLS • CG-DLS
Molar Mass • Size • Interactions • Charge

Introduction

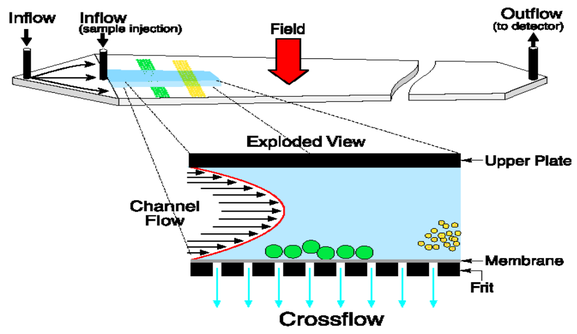
Measuring the molar mass and Size (Radius of Gyration (Rg) and Hydrodynamic Radius (Rh)) distributions of modified pectin samples by SEC has always been problematic as the shape of the pectin is both compositionally and molar mass dependent. Even worse, SEC has been shown to promote the formation of soluble aggregates during analysis[1].

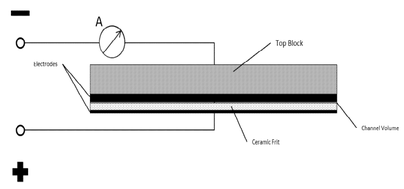
Obtaining electrokinetic (Electrophoretic mobility (μ), zeta potential,(ζ)) parameters whilst of obvious interest to explain some of the interactive behavior can likewise only be taken from the bulk material as it is traditionally only a batch measurement.

Here we demonstrate the combination of AF4 cross-flow induced separation and the application of an additional electric force field to produce a separation and to determine the pH dependant polarity of the molecules and to quantify electrokinetic parameters electrophoretic mobility μ and zeta potential ζ as a function of Rh.

Asymmetric Flow Field Flow Fractionation (AF4) and Electrical AF4 (EAF4)

Normal AF4 setup, Laminar Flow along a channel with height between 190 and 420um and a perpendicular flow through the membrane that induces a horizontal displacement related to Rh





Modified design introducing a variable electric field as a modifier on sample behaviour and separation

Theory in Brief

The electrophoretic mobility μ is defined within the following equation

$$v_{ep} = \mu \; E$$

Where v_{ep} is the migration velocity produced by the applied electric field and E is the electric field strength.

Instead of applying a given voltage as in electrophoresis, a specific current, *I is applied.* The absolute value of the electric field strength E between parallel electrodes is described as

$$E = \frac{U}{d} = \frac{R\,I}{d} = \frac{I}{A\,K}$$

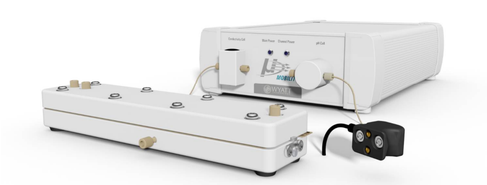
d is the distance between electrodes, R is the electrical resistance, A is the electrode area and K is the conductivity of the eluent.

In AF4, the retention time depends on the sample and the ratio of the cross flow to channel flow. In EAF4 this is modified by the drift velocity induced by the electric field.

$$v = v_{ep} + v_c$$

For more details please see the explanation sheet

Instruments



The experiments were performed on an HPLC consisting of an isocratic pump, an auto sampler and a variable wavelength detectorcoupled to a Wyatt DAWN HELEOS II MALS detector and a Wyatt Eclipse AF4 (with center downstream injection technique, together with a custom-made EAF4 channel

The EAF4 channel is an assembly of a top and bottom block, both made of PET, one ceramic frit, an O-ring (made of Viton or Kalrez), an ultra-filtration membrane (regenerated cellulose or polyethersulfone as an active layer) and a spacer foil (made of biaxial oriented polyethylene terephthalate) with 350µm thickness.

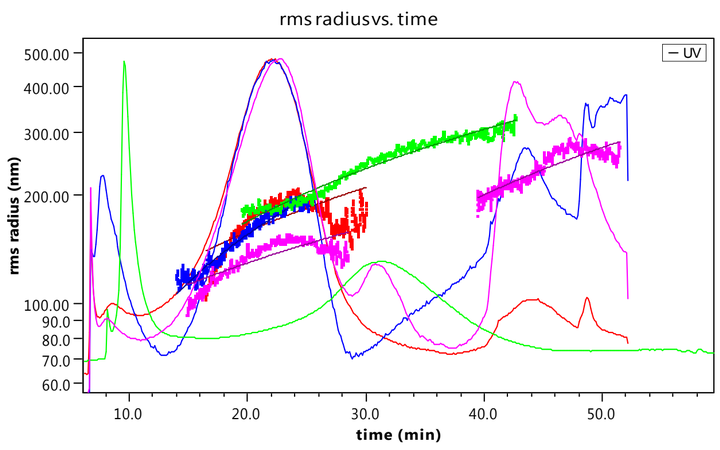
The electrodes were made of platinized stainless steel, opposing each other parallel in a symmetrical manner with a distance of 3.7 mm. One electrode was mounted on the top block, while the other electrode was placed below the ceramic cross-flow frit.

Results 1

4 pectin samples with different degrees of esterification (A (71%), B (74%) C (69%) D (30%) were examined coupling EAF4 and MALS [2].

The initial pure AF4 results showed the complexity of the samples as well as the presence of large aggregates.

Rg determined by MALS.



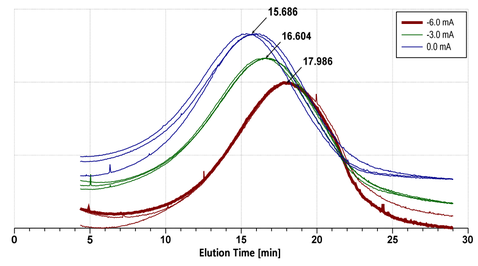
Comparison of rms radius versus time for four samples. A (red), B (blue), C (magenta), D (green).

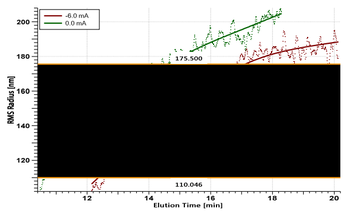
Results 2

The application of the electric field shows as a shift in the retention time and changes according to the strength of the field which is the migration velocity.

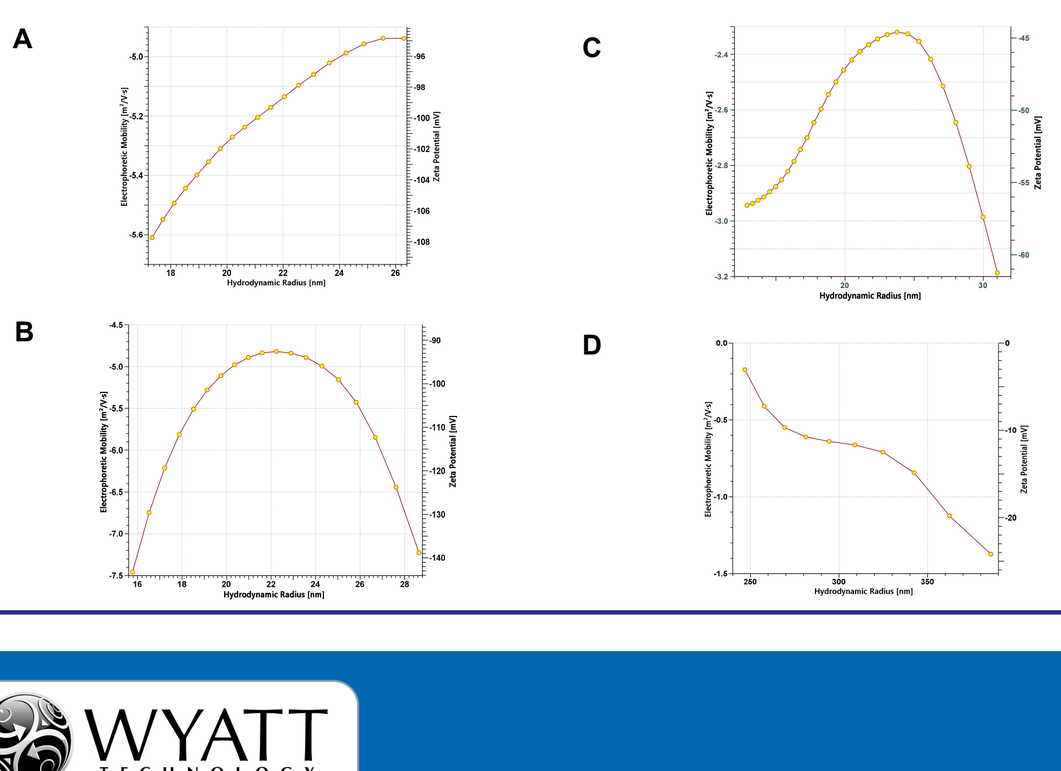
As the RMS radius is determined by MALS independently of the retention time we can use this to calculate the shift induced by the electric field.

The Rh according to retention time is then calculated using AF4 theory.





Results 3



Conclusion

The 4 samples are clearly differentiated in both terms of size and the charge each possesses. The ability to produce a separation and have a charge relationship shown for each part of the distribution means we can pick out trends that are not otherwise available.

Sample A, is a non-calcium sensitive pectin that shows a fairly simple decrease of μ *and* ζ with increasing size. The slope does fall off towards the upper end of the size range so it is possible that an inflection point would be reached for larger sizes.

Sample B, is a non-calcium sensitive pectin with an increased degree of esterification. This has induced a radical change in the charge properties in respect to size. In this instance the charge increases between Rh = 16 and 22nm and then decreases after this point.

Sample C is a calcium sensitive Pectin with 69% esterification. This sample has a definite charge inflection point around 24 nm but has a significantly lower overall charge characteristic than the first 2 samples.

Sample D has the lowest level of esterification (30%) and the lowest level of charge. The charge level drops with increasing size with an area in the middle of the distribution that shows a drop of in this change.

The challenge is now to relate these size:charge distributions to changes in the stability and behaviour of the samples.

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