Molecular mass characterization of tannins by organic A4F multidetection

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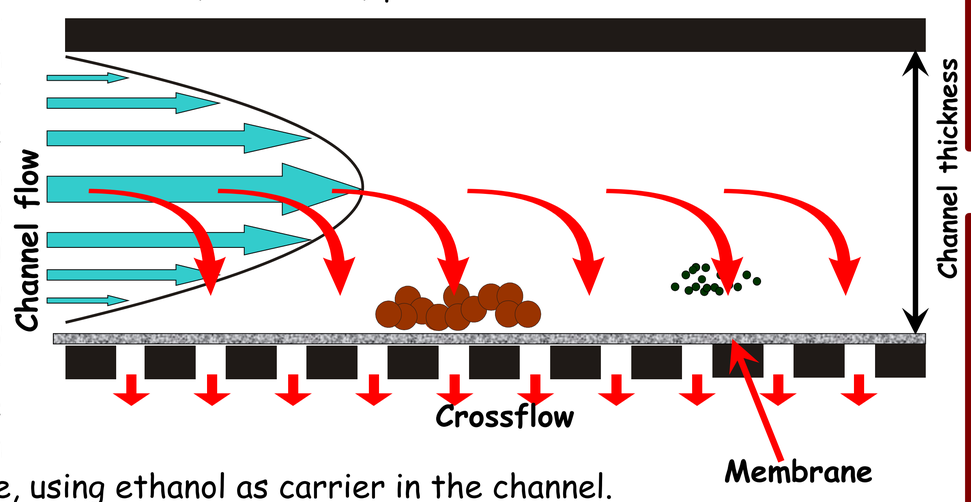
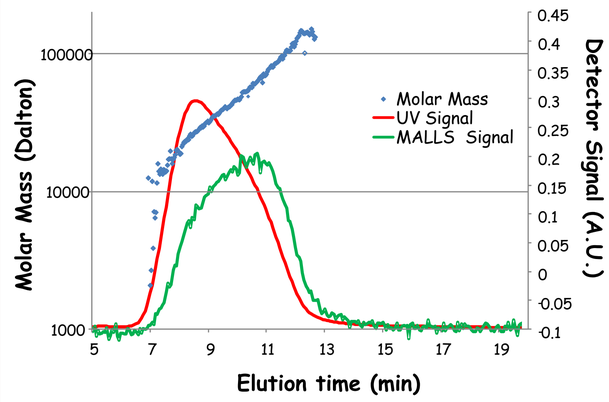
Tannins extracted from plants usually present a high **heterogeneity**. **Size distribution** has an impact on tannin properties, and its determination is an analytical challenge (irreversible adsorption on chromatographic support, calibration,…). Furthermore, some reactions such as oxidation of tannins make their structures more complex and have a major effect on their polymerization degrees and masses.

This work presents preliminary results about **Asymmetrical Flow Field Flow Fractionation** (A4F) in organic solvent hyphenated with UV and **Multi Angle Laser Light Scattering** (MALLS) detection. With this technique, no chromatographic support is required, minimizing problems owing to tannin adsorption. The use of MALLS allows the direct calculation of molecular weight and radii of gyration.

A4F is based on a liquid flow field in a semi-permeable channel. Because of the parabolic main flow profile, the macromolecules are **size-separated** (from the **smaller** to the **larger** ones) under a field of carrier solvent (named crossflow). MALLS associated to concentration detector (UV or DRI) permits to determine size and mass of analyzed macromolecules. One of the main advantages of A4F compared to SEC is the **lack of interactions** (in optimized conditions) between analytes and the membrane covering the channel permitting the elimination of dissolved part of the sample. A4F is used in this study in organic mode, using ethanol as carrier in the channel.



Typical UV and MALLS fractograms obtained from tannins in ethanol :



Analytical conditions :

Sample conc.: **0.5 mg.mL^{-1}**
Sample filtration at 0.22µm

A4F :
Detector flow rate : **0.5mL.min^{-1}**
Crossflow rate : **3mL.min^{-1}**
5kDa membrane
Injection : **50µL**

UV tuned at **280 nm**

Here masses are ranged from 10000Da up to 100000Da. The average molecular weight is about M_w = 4.351 10^4 g.mol^{-1}

Application to highly oxidized grape seed tannins

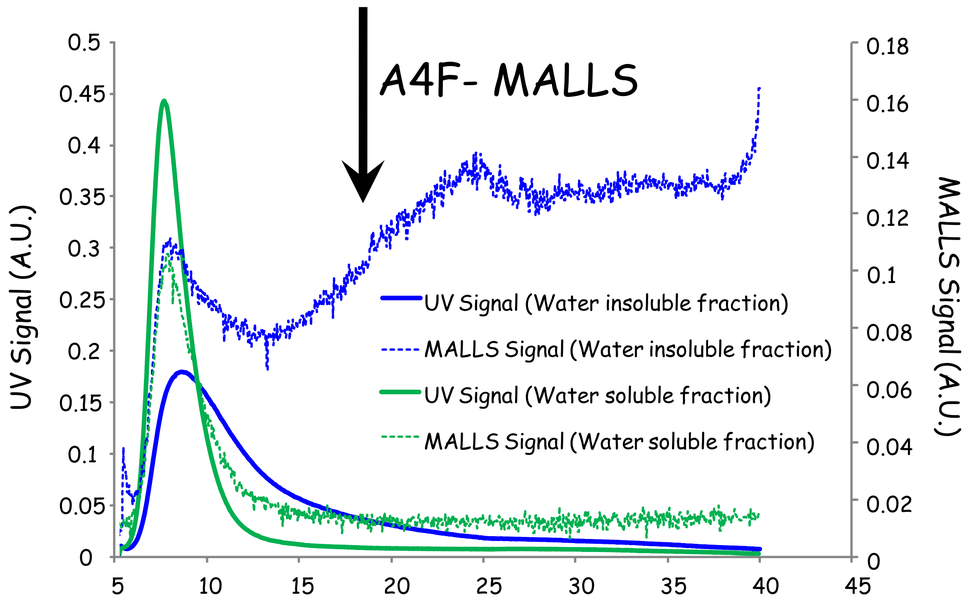
Whole oxidized grape seed tannin fraction (initial DP: 15)

Water soluble fraction

Water insoluble fraction

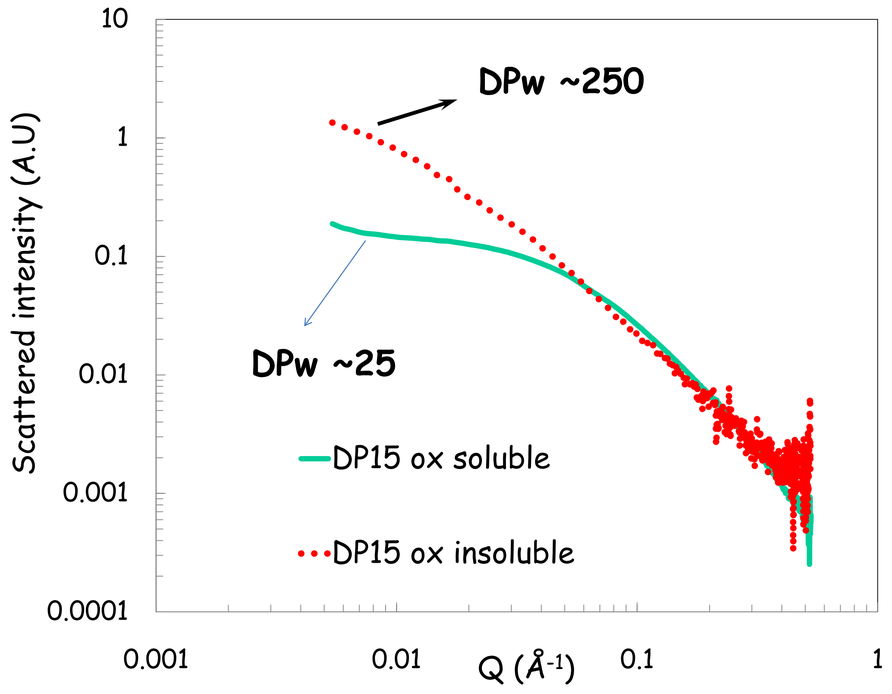
Fraction 1 Fraction 2 Fraction 3 — **A4F collected fractions**

A4F- MALLS



Fractograms of oxidized grape seed tannins in ethanol

Small Angle X-ray scattering



SAXS curves of water soluble and insoluble oxidized tannins

Thiolysis

Fraction	Degree of Polymerization	Thiolysis yield
Initial fraction	14.8	81.2
Whole oxidized fraction	14.2	35.8
Water soluble fraction	14.3	50.3

- Thiolysis results show no apparent differences between native and oxidized tannins. However, the reaction yields dramatically decrease, indicating that new oxidation bonds were formed (and that thiolysis results are not relevant in this case).
- A4F-UV-MALLS results illustrate the high degree of condensation of insoluble tannins. Actually, for insoluble tannins (blue curves), UV peak is highly tailing and MALLS signal starts to increase dramatically compared to soluble fraction (green curves). Insoluble tannins have a Mw about 10 times larger soluble ones.
- SAXS results show that both soluble and insoluble fractions have a higher DP than the initial DP (15), but insoluble species are much more polymerized.

Results obtained with **A4F-UV-MALLS** and **SAXS** are **consistent** and show the limitations of the thiolysis: increase of weight average molecular weight when oxidation occurs, insoluble fraction has larger DPs than soluble ones. This makes A4F – MALLS a promising tool to investigate the size distribution of tannins in **different solvents,** to determine **aggregate sizes**, but also to determine the **size distribution of complexes** formed between tannins and other **biomacromolecules.**

REFERENCE
Field Flow Fractionation Handbook, Martin E. Schimpf, Karin Caldwell, J. Calvin Giddings (2000). Wiley Interscience. Editors: John Wiley & Sons, Inc.

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