

Macromolecules and Secondary Metabolites in Grapevine and Wines, pp xxx-xxx, 2006

Tannins in hydroalcoholic solutions

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Introduction

Physico-chemical interactions involving polyphenols take a primary part in wine stability, clarification and taste. They are involved in the formation of hazes and precipitates [1], and affect the efficiency of the clarification and stabilization treatments applied to ensure wine clarity and stability [2, 3]. Polyphenol interactions with proteins, considered as responsible for the astringent perception of wine are also exploited in fining treatments [4, 5]. An improved understanding of these interactions is thus a key point to (i) elucidate the relationship between wine phenolic composition, organoleptic properties and colloidal stability; (ii) develop new processes and design low fouling-materials for wine elaboration and stabilization.

Previous studies demonstrated that grape seed and apple tannins constitute colloidal dispersions in hydroalcoholic solutions. The stability of these dispersions depends on the ethanol content and ionic strength of the solvent [6, 7]. This colloidal behavior of tannins is of primary importance in enology, as it influences their stability as well as their interaction with other macromolecules or surfaces. Grape seed tannin colloidal dispersions were thus further characterized through physical separation techniques coupled with UV absorption, dynamic light scattering (DLS) and small angle neutron scattering (SANS). DLS was used to evidence the formation of colloidal objects, to determine their mean hydrodynamic diameter and to monitor the stability of the suspensions. SANS allowed us to characterize the structure and the dimensions of the colloidal objects and the conformation of the dissolved macromolecules.

Materials and Methods

Tannin purification and characterization Tannins were extracted from freeze-dried seeds of *Vitis vinifera* and fractionated according to their mean degree of polymerisation in number (mDP) following the procedure described in [6, 7]. The

fraction used in the present study, analysed by Thiolyis [8] had a mDP of 11 and its percentage of galloylation was 27%.

Ethanolic and aqueous solutions Tannins were first dissolved in absolute ethanol, at a maximum concentration of 100 g/L. These solutions were then diluted with the solvent of interest to get the desired final composition (ethanol content and tannin concentration). Solvents were: (i) water simply acidified to pH 3.4 with hydrochloric acid (low ionic strength, in the order of 0.5 mM), (ii) a tartaric acid solution (2 g.L⁻¹) the pH of which was adjusted to 3.4 with NaOH (moderate ionic strength, in the order of 10 mM). Deuterated solvents (Euriso-top) were used for SANS experiments.

Preparation of colloidal dispersions Colloidal dispersions in water/ethanol mixtures were prepared by successive addition of small volumes of aqueous solvent in the tannin ethanolic solution (low supersaturation route), or by mixing of a small volume of the tannin solution in a large amount of aqueous solvent (high supersaturation route).

Dynamic light scattering Dynamic light scattering experiments were carried out using a Malvern Autosizer 4800 (Malvern Instruments, UK, multi-angle and APD detection) equipped with a 35 mW He-Ne laser ($\lambda = 633$ nm). The sample cell was thermostated at 25 ± 0.1 °C. The time-dependence of the scattered light was monitored and the autocorrelation function $G(t)$ of the colloidal objects measured. The diffusion coefficient D , derived from this autocorrelation function, was used to calculate their mean hydrodynamic radius R_H of the colloidal particles using the Stokes-Einstein equation.

Small angle neutron scattering Neutron scattering experiments were performed on the instrument D11 at the Institute Laue-Langevin. Scattering vector (Q) values ranged from $4 \cdot 10^{-3}$ to 4 nm⁻¹, corresponding to real space distances between 1500 and 1.5 nm.

Results

Phase diagram of the grape seed tannin fraction mDP 11 in water – ethanol solvents It is already known that procyanidins form colloidal dispersions in hydroalcoholic solutions having ethanol content within the wine ethanol percentage range [6, 7]. In the present study, we focused our interest on a given grape seed tannin fraction and performed systematic experiments according to the ethanol content (% v/v) and the ionic composition of the solvent.

Starting from a fully dissolved tannin solution in ethanol and adding aqueous solvents, dynamic light scattering and SANS experiments allowed us to evidence a succession of intermediate states including molecular solutions, metastable colloidal dispersions and precipitates. The relative locations of these intermediate states are displayed Figure 1 for the two studied solvents (ionic strengths 0.5 and 10 mM). In the “low supersaturation route”, acidified water was progressively added to the tannin solution in ethanol (composition A in the figure) to reach the composition C (ethanol 12% v/v, tannins 5 g.L⁻¹). SANS and DLS experiments were performed after each addition to evidence the threshold B for the formation of colloidal objects (route A to B to C). This threshold, evidenced by an abrupt rise in the scattered intensity, did not depend on the solvent ionic strength (Figure 2). By contrast, this ionic strength strongly influenced phase separation (see below “precipitation”).

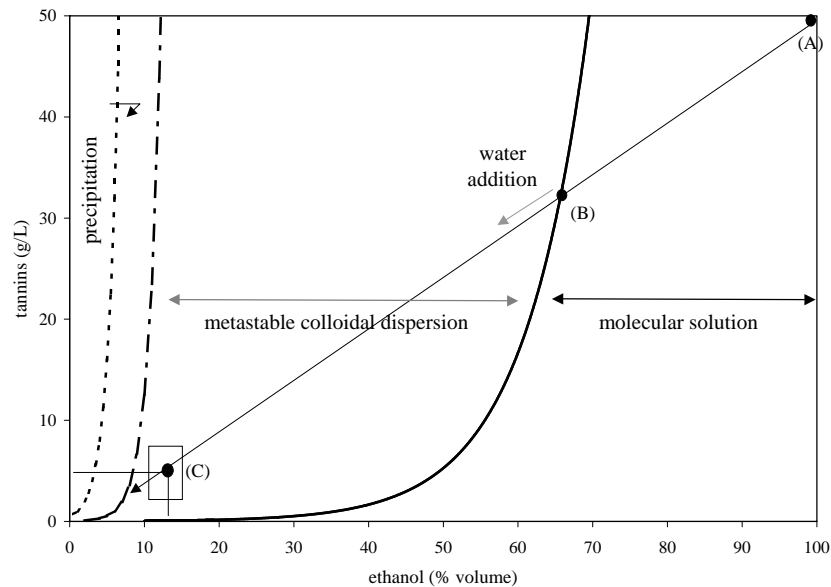


Figure 1. Thresholds for the formation of colloidal particles and precipitation for the tannin fraction in water/ethanol mixtures. Colloidal suspensions were prepared in both low (route A to B to C) and high supersaturation conditions. —: thresholds for the formation of colloidal particles; - - : thresholds for precipitation in the 10 mM ionic strength buffer, ···· : thresholds for precipitation in the 0.5 mM ionic strength buffer.

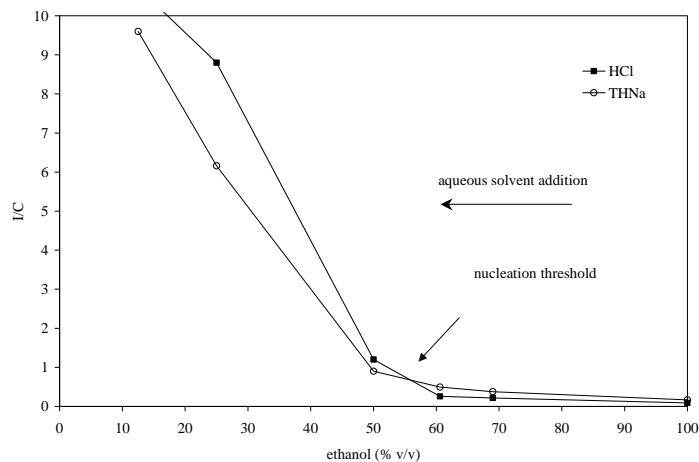


Figure 2. Rise in normalized scattered intensity observed by DLS upon progressively adding aqueous solutions to a tannin solution in absolute ethanol.

Colloidal dispersions The full SANS spectra of the colloidal dispersions were recorded for an ethanol content of 12 % and a tannin concentration of 5 g.L^{-1} . These spectra evidenced the co-existence of two distinct components: a steep power-law decay (Q^{-4}) at low Q ($< 0.01 \text{ \AA}^{-1}$), originating from the colloidal particles, and a slow

decay at high Q ($> 0.03 \text{ \AA}^{-1}$). Figure 3 shows the SANS spectrum of the sample formed under low supersaturation conditions. In these conditions the low Q -part of the spectrum could be fitted with the calculated scattering curve of polydisperse colloidal spheres. The curvature at the lowest Q values yielded the average radius ($R=337 \text{ nm}$, in the example show figure 3) and the polydispersity index (0.25 in the example) while the (Q^{-4}) decay observed at intermediate Q indicated that these particles have sharp interface. This sharp interface is consistent with the existence of a nucleation process when colloids were formed in low supersaturation conditions. The mean size of these colloids, in the order of a few hundred nm, depended on the supersaturation degree. In the case of a high initial supersaturation, bushy aggregates with a fractal dimension of 2.6 were formed instead of dense spheres.

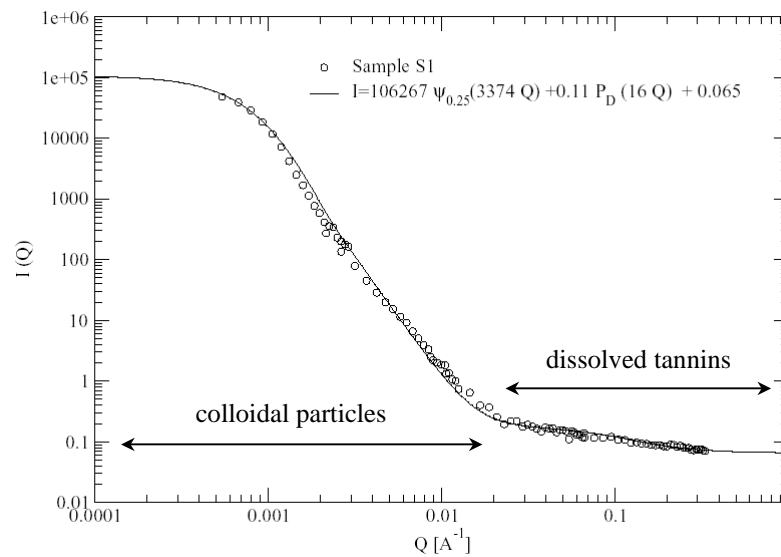


Figure 3 : Full SANS spectrum of the colloidal dispersion (ethanol 12%, tannins 5 g.L^{-1}) formed in low supersaturation conditions. $I(Q)$: scattered intensity; Q scattering vector.

The high Q part of the SANS spectrum was quite different from the scattering curve of large colloidal spheres (Figure 3). It could be fitted by the form factor for random coils with gyration radius $R_G = 1.6 \pm 0.5 \text{ nm}$. Using the scaling law for random coils, $R_G = a(N)^{1/2}$, and considering the mean degree of polymerisation $N = 11$ of the tannin fraction gives for the monomer size $a = 0.48 \text{ nm}$ which is consistent with the size of a monomer such as catechin or epicatechin [9]. The high Q part of the spectrum thus correspond to individual tannins in solution.

The above results indicate that there were at least two populations in the studied tannin fraction. A hydrophobic fraction, noted T1, is soluble only at very high ethanol content in the solvent ($>60 \%$); at lower ethanol contents, it forms colloidal objects. The other main fraction, noted T2, is more hydrophilic and remains in solution over a much wider range of ethanol contents. Tannin amounts in T1 were estimated: (i) from the ratio between the scattered intensities extrapolated at $Q = 0$ of the colloidal aggregates and the dissolved tannins; (ii) by ultracentrifugation

(elimination of the colloidal aggregates) at 100 000 g during 1 h and UV absorption measurements at 280 nm on the sample before and after centrifugation. These two evaluations led to a tannin amount involved in aggregation in the order of 2 %.

Precipitation Decreasing the ethanol content below 12% led to precipitation (figure 1). The DLS monitoring of the samples in 12 and 6 % ethanol are shown figure 5 for the two solvents. As stated before, the thresholds for precipitation were quite different in these two solvents. When precipitation occurred, ultracentrifugation and UV adsorption measurements were used to quantify tannin amounts in the precipitates. At low ionic strength (0.5 mM) phase separation only occurred for low ethanol contents (< 3% v/v in the wine tannin concentration range) and implied a very minor part of tannins. In the moderate ionic strength buffer (10 mM), phase separation occurred at ethanol contents below 8 % and the mass fraction implied was quite different. It ranged between 15% in 5% ethanol to 30% in 2% ethanol. Furthermore, this mass fraction remained constant when the tannin concentration was increased (between 1 and 4 g.L⁻¹). This indicates the existence of a third subpopulation in the fraction, the solubility of which is dependent on both the ethanol content and the ionic strength.

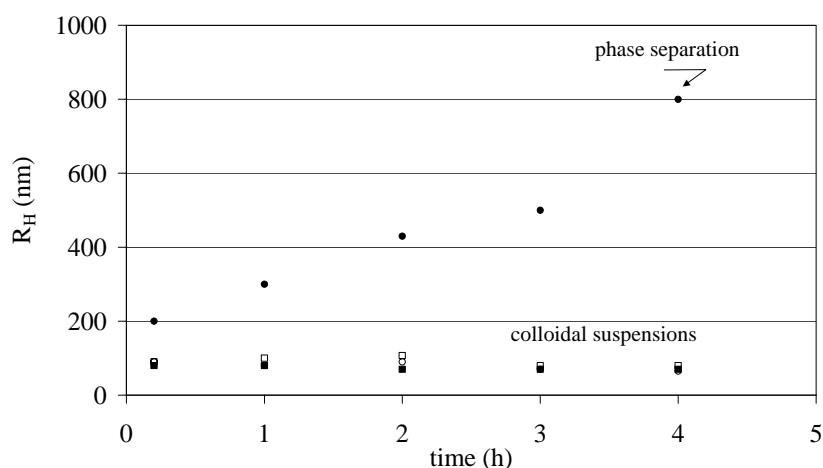


Figure 5 DLS monitoring of tannin colloidal suspensions in HCl (□, ■) and THNa (●, ○). Open symbols: 12% ethanol, tannin 5 g/L; black symbols : following to aqueous solvent addition to reach 6 % ethanol (tannins 2.5 g.L⁻¹). R_H: mean hydrodynamic diameter of the aggregates.

Conclusion This work allowed us to evidence three tannin populations of different physico-chemical behaviour in the studied tannin fraction (figure 6). The major population, called T2, is rather hydrophilic and comprises two sub-populations, T2a and T2b. Tannin structures in the T2a fraction remain soluble on a wide ethanol concentration range whatever the ionic content while macromolecules in the T2b fraction have a solubility that depends on both the ethanol content and the ionic strength. A minor part of the macromolecules in the tannin fraction (T1) exhibited a “hydrophobic” behaviour and was precipitated upon low aqueous solvent addition. This study evidenced the efficiency of coupling DLS and SANS approaches to study tannin colloidal behaviour and obtain structural information of both dissolved tannins and tannin colloidal aggregates. Further studies will have to be directed

towards the elucidation of the relationship existing between polyphenol structure and behaviour in solution.

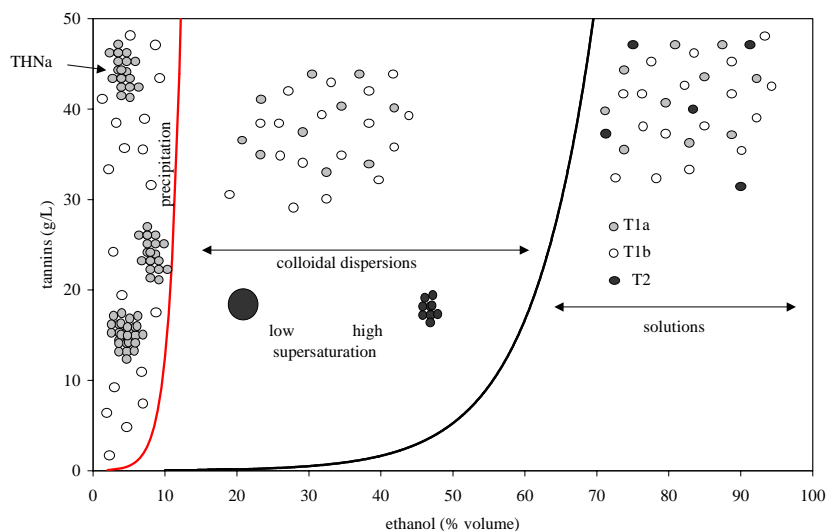


Figure 6 : colloidal behaviours of macromolecules of a grape seed tannin fraction in a 2 g.L^{-1} sodium tartarate buffer (moderate ionic strength).

Litterature cited

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