

# Characterization of (+)-Catechin–Acetaldehyde Polymers: A Model for Colloidal State of Wine Polyphenols

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The (+)-catechin–acetaldehyde condensation was studied as a model for tannin polymerization and precipitation in red wines. It was shown by using LSIMS that reaction products are catechin polymers with CH–CH<sub>3</sub> bridges at two reaction sites. Before the end of monomer conversion into polymers there is an aggregation followed by a phase separation. Particle mean diameter was measured by dynamic light scattering directly in reaction medium. Comparison between particle and polymer sizes showed that polymers aggregate into colloidal intermediates before a large polymerization degree is reached. These particles then coagulate further, which leads to polymer precipitation. This colloidal behavior is explained by hydrophobic forces since aggregates and precipitate redissolve in ethanol.

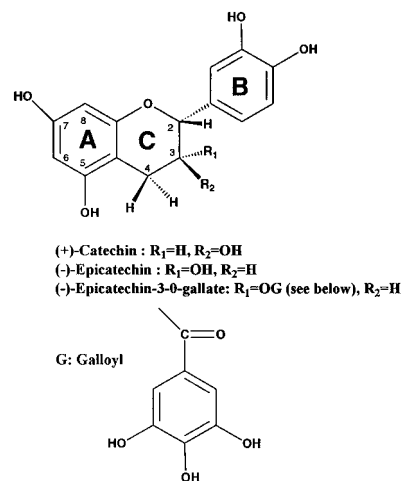
**Keywords:** Tannin; wine aging; colloid; catechin; acetaldehyde

## INTRODUCTION

The main phenolic compounds of young red wines are condensed tannins (procyanidins) and anthocyanins. They are natural grape components and are especially present in seeds (only tannins) and skins. Anthocyanins are responsible for the bright red color of young wines and tannins for their taste. Procyanidins are polymers with a structure consisting of (+)-catechin and (–)-epicatechin units (Figure 1) linked by C<sub>4</sub>–C<sub>6</sub> or C<sub>6</sub>–C<sub>8</sub> bonds and sometimes esterified by gallic acid on the epicatechin moieties (Prieur *et al.*, 1994).

During wine aging the polyphenolic content of wine becomes progressively more complex. Condensation degree and hydrophobicity of polyphenols affect taste, color, and colloidal stability (Haslam *et al.*, 1993). The average mass number of polyphenols measured in different wines was contained between 895 and 3750 (Ribéreau-Gayon and Glories, 1971). Thus, polymerization degree of polyphenols in wine does not seem to increase dramatically but precipitations do occur, indicating structural changes leading to decreasing solubility. In fact, it seems that during wine aging both depolymerization and condensation occur. Cleavage of interflavan bonds of procyanidins could happen at the pH of wine (Haslam, 1980). In addition, direct or indirect condensation between flavanols is believed to take place.

The effect of polyphenol transformations on wine color has been particularly studied (Liao *et al.*, 1992). Evidence of condensation occurrence in red wines is the continuous disappearance of free anthocyanins, which are progressively replaced by new polymeric colored compounds (Jurd, 1969; Somers, 1971). Their structures remain unknown. Nevertheless, several hypotheses have been advanced: One is the formation of an



**Figure 1.** General structure of monomeric flavan-3-ols.

orange xanthylium-based polymer (Jurd and Somers, 1970) by self-flavan-3-ol condensation. More recently, a new orange pigment has been identified in red wine (Cameira dos Santos *et al.*, 1996), which was shown to be a condensation product between an anthocyanin and a vinyl phenol (Fulcrand *et al.*, 1996a).

Indirect condensation involving acetaldehyde (Trillat, 1907; Timberlake and Bridle, 1976) could concern both anthocyanin–tannin and tannin–tannin condensation (Fulcrand *et al.*, 1996b; Es-Safi *et al.*, 1996) by CH<sub>3</sub>–CH linkages (Figure 2). Acetaldehyde in wine is produced biochemically by yeast metabolism during fermentation (Romano *et al.*, 1994). Another method of production of acetaldehyde is oxidation of ethanol by phenolic compounds (Wildenradt and Singleton, 1974) in the presence of oxygen.

In this paper, we study the case of indirect condensation linked to tannin–tannin polymerization with acetaldehyde. We use (+)-catechin–acetaldehyde condensation in a winelike model solution.

## MATERIALS AND METHODS

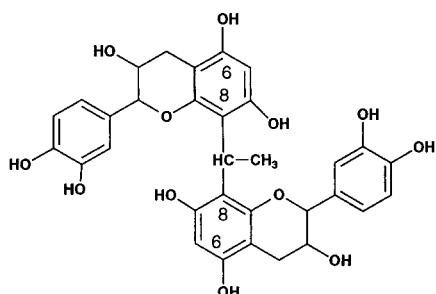
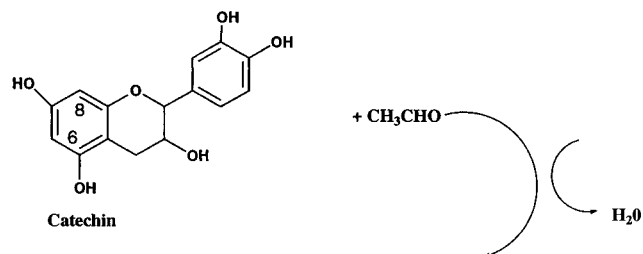
**Reagents.** All chemicals were of reagent grade. Model wine solution used was 12% (v/v) ethanol buffered to pH 3.2

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**Figure 2.** Reaction between catechin and acetaldehyde in acidic solution [adapted from Timberlake and Bridle (1976)].

(5 g/L tartaric acid, NaOH N for pH 3.2). Catechin was purchased from Extrasynthèse (Ref 952, Genay, France).

**Model Systems.** (+)-Catechin at different concentration levels ( $3.46 \times 10^{-3}$ ;  $8.65 \times 10^{-3}$ ;  $1.73 \times 10^{-2}$ ;  $3.46 \times 10^{-2}$  mol L<sup>-1</sup>) was mixed to ensure a fixed excess of acetaldehyde (0.45 mol L<sup>-1</sup>) in the model wine solution at 35 °C. A stock solution of acetaldehyde was used for all samples to ensure reproducibility.

**Kinetic Measurement.** Disappearance of (+)-catechin was monitored by HPTLC-silica 60 with ethyl acetate–formic acid (98:2) as eluent. Each plate had six (+)-catechin internal standards. Evaluation was carried out by using a TLC scanner II (Camag) with a deuterium lamp at 280 nm.

**Light Scattering Measurement.** Light scattering measurement was performed by using a ionized krypton laser (Spectra Physics 2020-11) with a wavelength of 6471 Å. An Amtec TFL detector was connected to a numeric correlator (BI 2030, Brookhaven Instruments Corp.). Dynamic light scattering (for mean particle size analysis) and static intensity (for turbidity estimation) were measured at 90° from incident beam.

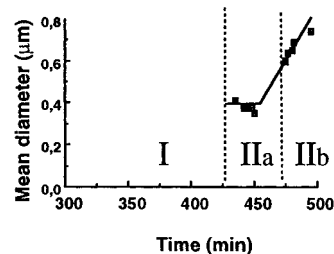
**Gel Permeation Chromatography.** GPC analysis was performed on peracetate derivatives (Williams, 1983) with a set of three TSK columns (2000H, 3000H, and 4000H) connected in series. Detection was at 280 nm. The system was calibrated with 13 polystyrene standards (312 Da–109 kDa).

**Mass Spectrometry.** LSIMS spectra were obtained using a Fisons Instruments Autospec-Q as follows: source, cesium gun in negative mode; calibration, cesium iodide between 200 and 3000 Da; matrix, glycerol; bombardment energy, 35 keV, 2 μA; temperature, <40 °C; sample solvent, anhydrous methanol.

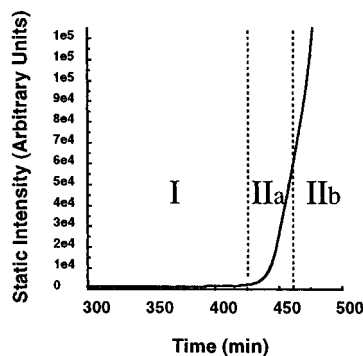
## RESULTS AND DISCUSSION

To speed the reaction rate, a fixed excess of acetaldehyde (0.45 mol L<sup>-1</sup>) compared to (+)-catechin concentrations (between  $3.46 \times 10^{-3}$  and  $3.46 \times 10^{-2}$  mol L<sup>-1</sup>) was used. This also avoids side reactions because the condensation process is much faster in the presence of acetaldehyde (Bakker *et al.*, 1993). The disappearance of (+)-catechin followed a first-order kinetic law. The kinetic constant value, measured at 35 °C, was the same ( $K = 0.21 \pm 0.05$  h<sup>-1</sup>) for all (+)-catechin concentrations tested (Saucier *et al.*, 1996).

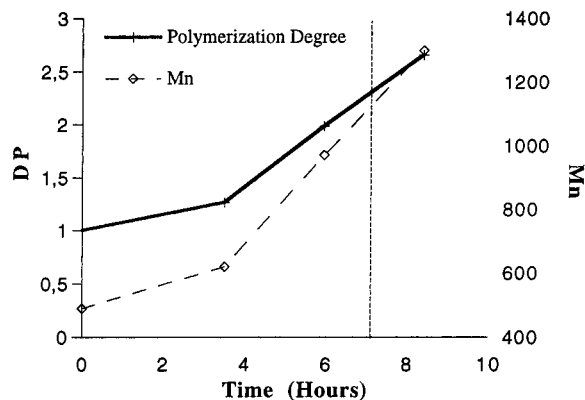
During reaction, a haze developed in the solution which became with time a precipitate. Scattered light



**Figure 3.** Mean particle diameter measured during reaction [no measure was possible in part I because the intensity was too low (see Figure 4)].



**Figure 4.** Static intensity measured at 90° during reaction (this measure permits a turbidity estimation).

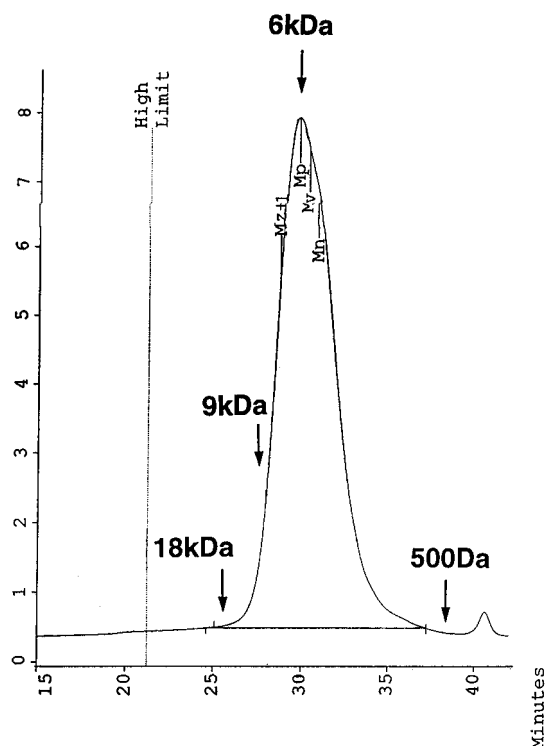


**Figure 5.** Number-average mass of polymers before and after phase separation (dashed line), measured by GPC.

intensity and mean particle size were measured directly in reaction medium by light scattering measurement with a laser source. For all (+)-catechin concentrations tested, static intensity measured during reaction showed two parts. An example of typical results is illustrated with the case of  $8.6 \times 10^{-3}$  mol L<sup>-1</sup> for initial catechin concentration (Figures 3–5).

In the first part of reaction (Figure 3I), static intensity was very low and constant and no particle size could be measured (Figure 4I). Quite suddenly (Figure 3IIa), the static intensity rose and particles with average diameters close to 4000 Å were detected (Figure 4IIa).

In the last part of the reaction (Figures 3IIb and 4IIb), particle size and static intensity rose, indicating a particle association into larger aggregates. Average masses were measured by GPC before and after phase separation (Figure 5). The results show that the apparition of particles is not due to a sudden rise in polymerization degree or to high polymer forms. Like all condensation polymerization reactions, the average molecular weight increased steadily. The polymer mass of precipitate was recovered at the end of phase separation (IIb, 24 h) and studied by GPC. Although the use



**Figure 6.** Mass distribution of peracetate derivatives of the precipitate. No monomer ( $m = 500$ ,  $R_t = 38.4$ ) is present. Parameters extracted from the curve were  $M_p = 6320$ ,  $M_n = 4503$ ,  $M_w = 5562$ , polydispersity = 1.235).

of polystyrene standards is a limitation in mass precision, this technique permits an estimation of average

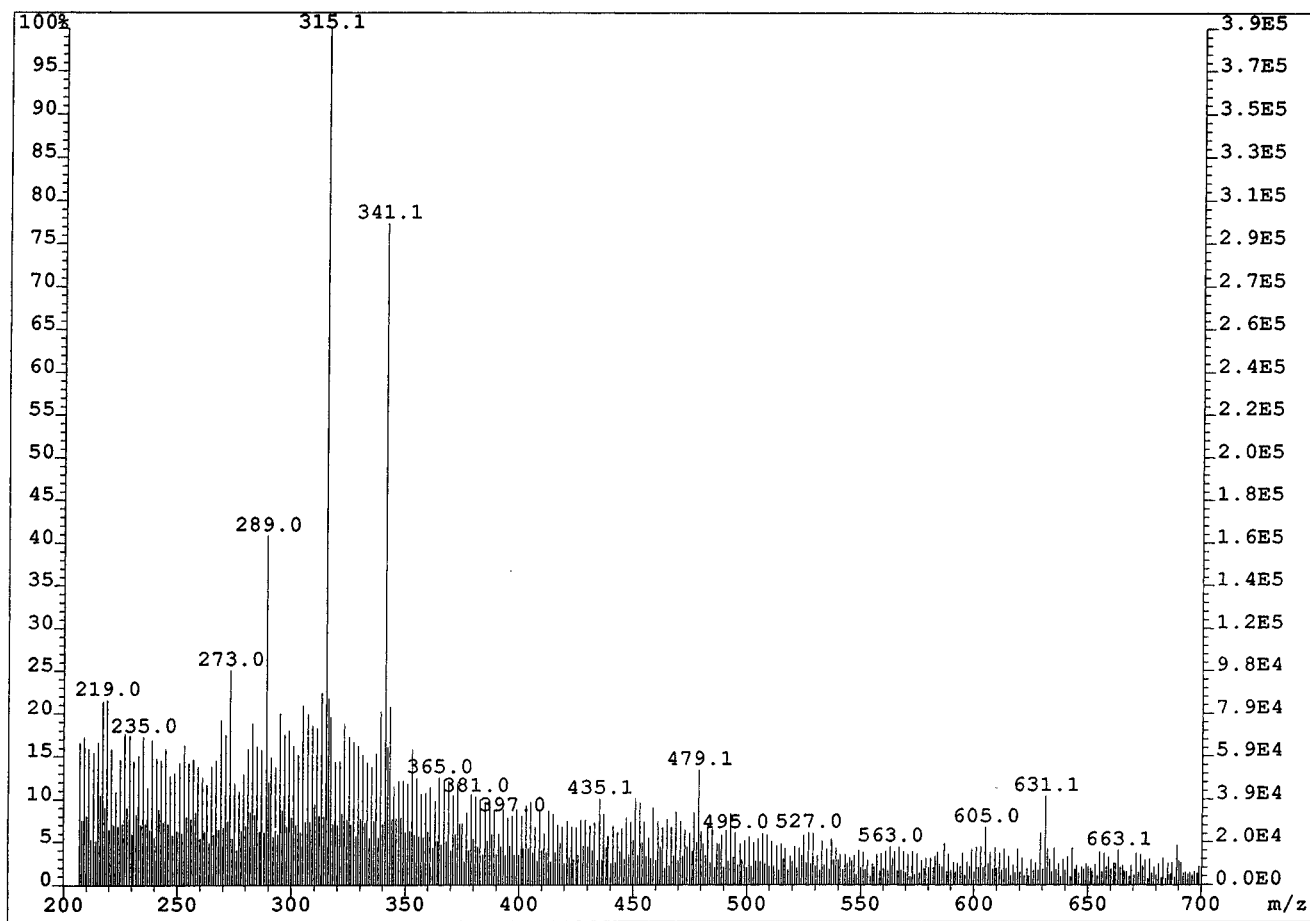
mass and mass distribution. The curve (Figure 6) shows a broad unimodal distribution with masses ranging from 1 000 to 18 000 Da. The peak mass is close to 6 000, which corresponds to a dodecamer peracetate (equivalent to 3 600 of the native polymer). The size of a polymer with a mass of 3 600 is close to 36 Å (Flory, 1969). The more relevant fact to note here is that all polymer sizes are about a hundred times smaller than the observed particle diameters.

Thus, particles observed by light scattering are constituted by an aggregation of a very large number of small polymers. This self-association process is similar to a critical micellar concentration (cmc; Saucier *et al.*, 1996). Nevertheless, the particle sizes observed (near micrometer) are much larger than micelles. The term critical aggregation concentration (cac) would be more appropriate in our case. This critical concentration decreases with polymer size due to hydrophobic interactions. Indeed, the precipitate is ethanol soluble but water insoluble.

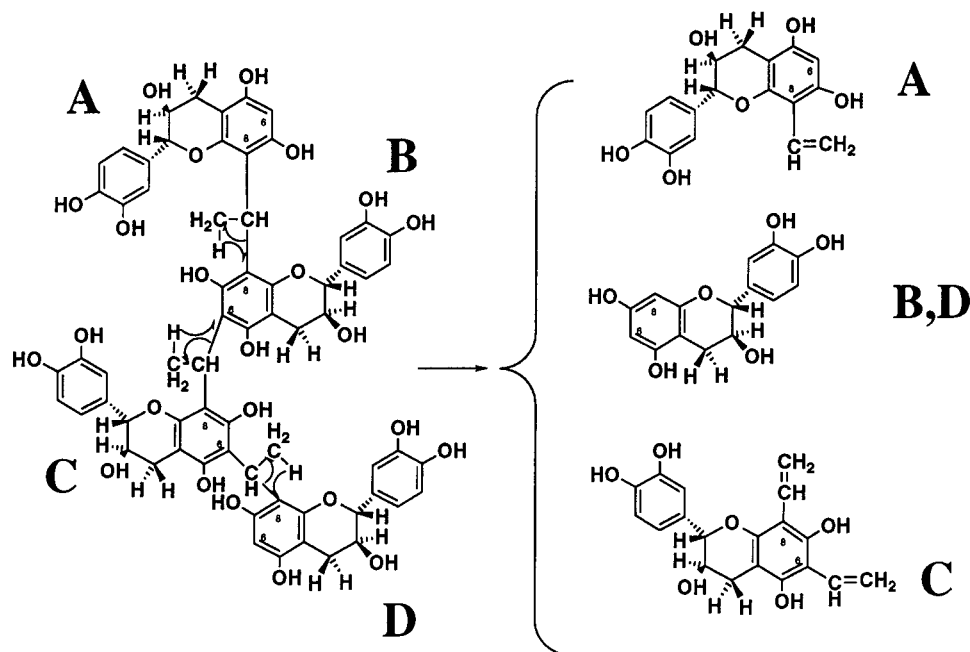
Concerning the chemical structure of the polymer, it has been shown previously (Dournel, 1985) by  $^1\text{H}$  NMR that only the A ring (Figure 1) of (+)-catechin was involved in acid-catalyzed acetaldehyde condensation. This is in accordance with the  $\text{CH}-\text{CH}_3$  bridging model of Timberlake and Bridle, which was confirmed by FAB-MS for malvidin-catechin dimer (Archier, 1992).

We have used LSIMS in negative mode to investigate the structure of the polymer precipitate. The spectra (Figure 7) show a relatively small dimer ion peak at  $m/z$  605 corresponding to deprotonated dimer of two catechins linked by an ethyl bridge.

The most intense peaks can be observed at  $m/z$  289,



**Figure 7.** LSIMS spectra obtained in negative mode by direct injection of the precipitated polymers.



**A**: 8-Vinyl-Catechin  $m/z = 315$ ,  $[M-H]^-$ ; **B,D**: Catechin  $m/z = 289$ ,  $[M-H]^-$

**C**: 6-8 di-Vinyl-Catechin  $m/z = 341$ ,  $[M-H]^-$

**Figure 8.** Fragmentation process observed in LSIMS experiments (see Figure 6).

315, and 341, which are molecular weights of monomeric forms. At first sight this seems to contradict the absence of monomer indicated by GPC results. In fact, this is proof for a fragmentation mechanism (Saucier *et al.*, 1995) (Figure 8) which leads to vinyl-catechin ( $m/z$  315,  $[M-H]^-$ ) and divinyl-catechin ( $m/z$  341,  $[M-H]^-$ ) fragments. The absence of a trivinyl-catechin fragment shows that (+)-catechin is bifunctional under our experimental conditions. In fact, the fragmentation observed is similar to the process of depolymerization already observed with this type of compound in acidic solution (White and Foo, 1990; Tanaka *et al.*, 1994).

Other polymeric forms, up to vinyl hexamer and vinyl octamer ( $m/z$  2211 and 2528, data not shown) were detected by this technique. Nevertheless, the ion intensities were decreasing with increasing molecular weight (100 times less intense for vinyl octamer than vinyl-catechin). LSIMS does not permit us to determine the relative abundance of each species or to determine the average molecular weight due to fragmentations (Vivas *et al.*, 1996) and mass range limitation. GPC is here the only method to estimate correctly average mass and polymerization degree during the reaction.

## CONCLUSION

Products from (+)-catechin-acetaldehyde reaction are  $CH-CH_3$ -bridged catechins probably in the 6- and 8-positions of the A ring (Figure 1). LSIMS provides specific fragments ( $m/z$  289, 315, and 341) for these polymers.

Hydrophobicity and behavior of these compounds should be further studied. The nonspecific association process observed is likely to occur with other polyphenolic compounds such as wine procyanidins. The intensity of association depends on polyphenol concen-

tration and hydrophobicity. The influence of carbohydrates such as proteins (Siebert *et al.*, 1996) and polysaccharides on polyphenol colloidal stability should be considered. This would help us to understand the stability of wine polyphenols in relation to clarity, longevity, and organoleptic properties.

## ABBREVIATIONS USED

DP, mean degree of polymerization; ES, electrospray; FAB, fast atom bombardment; GPC, gel permeation chromatography; HPTLC, high-performance thin layer chromatography; LC, liquid chromatography; LSIMS, liquid secondary mass spectrometry; MS, mass spectrometry;  $M_p$ , peak mass;  $M_n$ , number-average mass;  $M_w$ , weight-average mass;  $R_t$ , retention time.

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