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HPLC Retention Thermodynamics of Grape and Wine Tannins

Jennifer A. Barak and James A. Kennedy*

Department of Viticulture and Enology, California State University, MS VR89, 2360 East Barstow Avenue, Fresno, California 93740-8003, United States

ABSTRACT: The effect of grape and wine tannin structure on retention thermodynamics under reversed-phase high-performance liquid chromatography conditions on a polystyrene divinylbenzene column was investigated. On the basis of retention response to temperature, an alternative retention factor was developed to approximate the combined temperature response of the complex, unresolvable tannin mixture. This alternative retention factor was based upon relative tannin peak areas separated by an abrupt change in solvent gradient. Using this alternative retention factor, retention thermodynamics were calculated. Van't Hoff relationships of the natural log of the alternative retention factor against temperature followed Kirchoff's relationship. An inverse quadratic equation was fit to the data, and from this the thermodynamic parameters for tannin retention were calculated. All tannin fractions exhibited exothermic, spontaneous interaction, with enthalpy−entropy compensation observed. Normalizing for tannin size, distinct tannin compositional effects on thermodynamic parameters were observed. The results of this study indicate that HPLC can be valuable for measuring the thermodynamics of tannin interaction with a hydrophobic surface and provides a potentially valuable alternative to calorimetry. Furthermore, the information gathered may provide insight into understanding red wine astringency quality.

KEYWORDS: *proanthocyanidin, tannin, grape, wine, thermodynamics, enthalpy, entropy, interaction*

INTRODUCTION

Tannins are a group of plant-based phenolic natural products that are abundant in the plant kingdom.[1] As such, they are present in many foods and beverages, including red wine, and are important with regard to food quality and human health.[2−4] Red wine tannins are composed almost exclusively of flavonoid-based proanthocyanidins (Figure 1A) extracted from grapes during wine production.[5,6] These extracted tannins undergo significant structural change following biosynthesis in the plant and through the course of wine production and aging (Figure 1B); the nature of these changes is of perennial interest to chemists due to their complexity and sensory importance.[7−12]

There is general interest in understanding tannin structure and developing analytical methodologies for tannins in foods and beverages. This is primarily due to their impact in the areas of human health, food processing, and sensory properties.[13−18] From a health standpoint, tannins may be both beneficial and detrimental.[3]

Tannin activity is related to surface chemistry and the ability of tannins to noncovalently associate with proteins and other macromolecular structures.[17,18] In red wines, tannin activity is considered to be an essential feature of overall wine quality, tannins being responsible for astringency. The perception of astringency is a tactile sensation generally attributed to increased friction in the oral cavity as a result of interaction and precipitation of lubricating salivary proteins with tannins and/or binding to oral epithelial cells.[2,3,19] The process of tannin precipitation of salivary proteins can be described as a three-stage process (Figure 1C). Data from tannin−protein interaction studies are consistent with hydrophobic interaction and hydrogen-bonding involvement.[20−26]

Red wine astringency is often described using qualitative terms that range from 'velvety' and 'soft' to 'harsh' and 'unripe'.[27] Potential explanations for the differences in astringency descriptors vary and include relative tannin concentration, wine matrix composition, and tannin structure.[28] Tannin concentration and matrix components such as ethanol, sugar, and wine acidity have all been shown to influence astringency perception.[29,30] Sensory studies have also shown that tannin structure (e.g., subunit composition, average degree of polymerization) have an impact on astringency.[17,18] Given that tannin structure has been shown to affect astringency and is anecdotally considered to be responsible for red wine astringency quality, there is a need to develop analytical methodologies that measure structure−activity relationships. The ability to routinely measure the interaction of tannins with macromolecules is considered to be a critical need.

Recent developments in our understanding of tannin−macromolecular interaction suggest that measuring the thermodynamics of this interaction may provide useful information.[20−23,31−33] A recent study by McRae et al.[33] found that tannin structure modification affected the thermodynamics of tannin−peptide interaction when measured by isothermal titration calorimetry (ITC). Specifically, the study found that tannin "age" was associated with a reduced enthalpy of interaction with poly-L-proline, a model peptide. Moreover, the results from ITC experiments conducted at various temperatures were consistent with initial tannin−peptide interaction being dominated by hydrophobic interactions (Figure 1C). Given that salivary proteins have an abundance of proline-rich proteins[34] and proline-rich motifs have an extended hydrophobic surface for interaction,[35] the results from McRae et al. suggest that hydrophobic interaction

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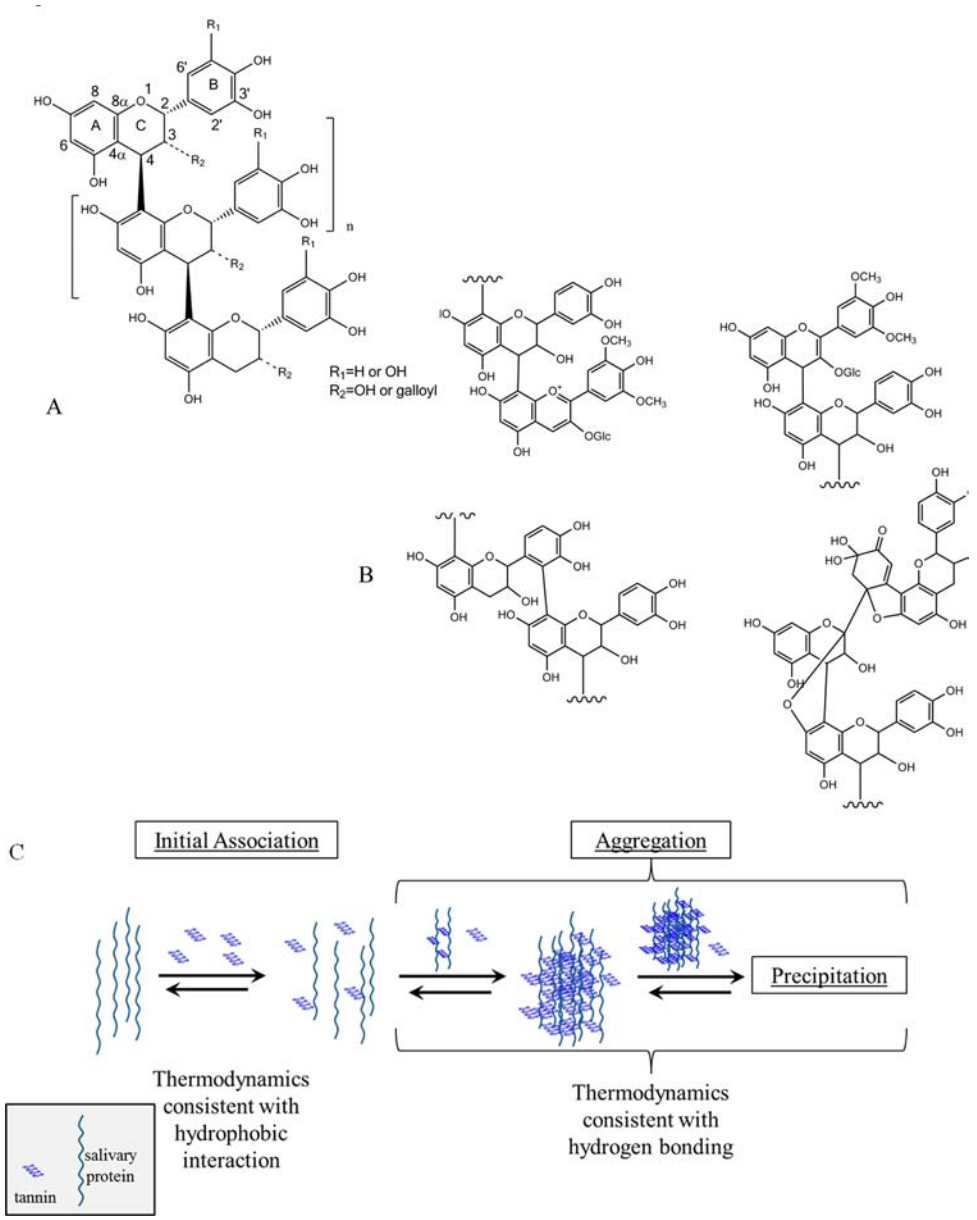

Figure 1. (A) Generalized structure of proanthocyanidins. (B) Examples of tannin structural features that are generally considered to form as a result of age in red wines and are a result of acid-catalyzed and oxidation reactions.[9,12] (C) Three-stage model (initial association, aggregation, and precipitation), based upon isothermal titration experiments,[33] of tannin interaction with salivary proteins, indicating hydrophobic and hydrogen-bonding interactions and with the theoretical reversibility of individual stages shown.

is significant in tannin−salivary protein interaction. Using this as a basis, the goal of this study was to develop a reversed-phase HPLC method that could be used for measuring the thermodynamics of interaction between tannins and a hydrophobic surface. This technique is based upon the reversed-phase response of analytes to temperature[36] and has been successfully used for measuring the thermodynamics of retention.[37,38]

EXPERIMENTAL PROCEDURES

Chemicals. All solvents were of HPLC grade. Acetone, acetic acid, acetonitrile, L-(+)-ascorbic acid, hydrochloric acid, lithium chloride, methanol, phosphoric acid, *N*,*N*-dimethylformamide, and sodium acetate anhydrous were purchased from VWR International (Radnor, PA, USA). Phloroglucinol, (−)-epicatechin, and trifluoroacetic acid (99+% spectrophotometric grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Tannins were prepared from grapes and wines produced in California. Grapes sourced from the university vineyards (*Vitis vinifera* L. cv. Cabernet Sauvignon) were harvested while still green, manually separated into skin and seed components, and stored at −80 °C until extraction, purification, and fractionation. Grapes were harvested while still green to minimize tannin degradation. Wines were either purchased from a wine shop or acquired directly from the winery and included three vintages of Cabernet Sauvignon (CS2012, CS2010, CS1990) produced by the same winery (Napa Valley, CA, USA).

Extraction and Preparation of Seed, Skin, and Wine Tannins. Frozen seed and skin tissue samples were removed from −80 °C storage and extracted and processed similarly to previously published methods.[39] Briefly, tissues were extracted in 70% v/v acetone for 24 h, concentrated under reduced pressure at 40 °C to remove acetone, and then lyophilized to a dry powder. For purification and fractionation, seed and skin powders were dissolved in 60% v/v methanol containing 0.05% v/v trifluoroacetic acid (TFA) and then applied to a glass column (Michel-Miller, Vineland, NJ, USA) containing Sephadex LH-

20 chromatography resin (Amersham, Uppsala, Sweden) to a bed volume of 290 mL (40 × 230 mm) and equilibrated with 60% v/v methanol containing 0.05% v/v TFA. Step gradients and relative elution volumes were consistent with that described previously.[39] The initial elution solvent (60% v/v methanol) was discarded as it contained low molecular weight phenolics in addition to low molecular weight nonphenolic impurities. In addition, the fraction eluted with 60% v/v acetone was discarded as it contained very little material. Elution solvents were selected to produce tannin fractions of increasing molecular mass.[39] Eluted tannin fractions were concentrated under reduced pressure at 35 °C to remove organic solvent, and the aqueous portion was lyophilized to a dry powder and stored at −4 °C.

Wine samples (750 mL) were prepared similarly, although the wine was applied directly to the same Sephadex LH-20 containing column and was also washed with 50% v/v methanol containing 0.05% v/v TFA to elute additional low molecular weight phenolic compounds. Otherwise, the preparation of individual tannin fractions was the same as that of the seed and skin samples. A total of five tannin fractions for each grape and wine sample was used for subsequent analysis.

Instrumentation. All experiments were conducted on an Agilent 1260 (Santa Clara, CA, USA) HPLC system. This system consisted of a model G1331B pump and degasser, a G1329B autosampler, a G1315C DAD/UV−vis detector, a G1316A column heater, and a system controller. Data processing was performed using ChemStation for LC 3D systems version B.04.03.

Acid Catalysis in the Presence of Excess Phloroglucinol (Phloroglucinolysis). Phloroglucinolysis was used to characterize seed, skin, and wine tannin samples, according to the method of Kennedy and Jones,[40] using a modified HPLC method.[39] For quantitation of subunit products, the method of Kennedy and Jones was also utilized, using (−)-epicatechin as a standard.[40]

Gel Permeation Chromatography (GPC). The 50% elution times for seed, skin, and wine tannins were determined by GPC, using the method of Kennedy and Taylor.[39] One change to the published method was that a 10^3 Å column was used instead of the 100 Å column to allow for elution time determination of higher molecular mass samples. For all samples, 40 μg injections were made.

Reversed-Phase Chromatography To Determine Thermodynamics of Interaction. The HPLC method for measuring the thermodynamics of tannin interaction utilized a rigid spherical polystyrene divinylbenzene reversed-phase column (PLRP-S, 2.1 × 50 mm, 100 Å, 3 μm, Agilent Technologies) protected with a guard column (PRP-1, 3 × 8 mm, Hamilton Co., Reno, NV, USA). Tannin isolates (5 mg/mL) were dissolved in an aqueous solution containing methanol (15% v/v), 40 mM sodium acetate, and 20 mM HCl. Prepared tannin-containing solutions were filtered using a 0.45 μm PTFE syringe filter (Grace Davison Discovery Scientific, Deerfield, IL, USA) prior to injection (35 μg). The mobile phases were 1.5% (w/w) H_3PO_4 in water (180 mM, mobile phase A) and 20% (v/v) mobile phase A in acetonitrile (B) with a flow rate of 0.21 mL/min. The linear gradient was as follows (time in min (%B)): 0 (14%), 18.0 (34%), 18.0−19.0 (34%), 21.5 (70%), 21.5−24.0 (70%), and 24.0−28.0 (14%). This method was based upon a previously published method[41] with modifications designed to reduce the run time.

To obtain thermodynamic information, samples were run at eight column temperatures (25−60 °C, 5 °C increments). For thermodynamics calculations, all temperatures were converted to Kelvin to report thermodynamic parameters in SI units. DAD detection was at 280 and 520 nm. Chromatograms were integrated as follows (Figure 2A): For total tannin ($Tannin_T$), chromatograms were baseline integrated from 5 to 28 min, excluding resolved peaks in the 5−24 min region. For partial tannin ($Tannin_P$), the peak area for tannin eluting from 24 to 28 min was determined.

Data Analysis. The thermodynamics of retention was determined using $Tannin_T$ and $Tannin_P$ peak area information gathered at the eight column temperatures. For each chromatogram, an alternative retention factor for the tannin (k_{alt}) was calculated.

$$k_{alt} = \frac{tannin_T}{tannin_T - tannin_P} \quad (1)$$

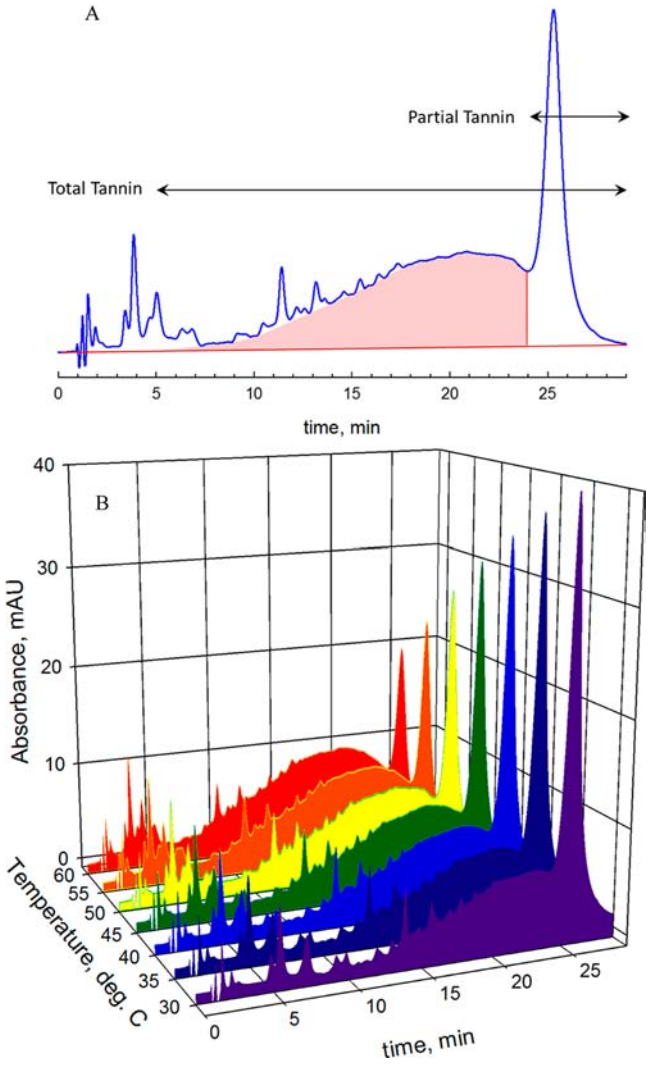

Figure 2. (A) Integrated chromatogram indicating partial ($Tannin_P$) and total tannin ($Tannin_T$) peak area integration. (B) Response of purified grape seed tannin to temperature.

From this, ln k_{alt} values for each sample at the eight temperatures were plotted against their corresponding temperature, and an inverse quadratic equation (eq 2) was fit to the experimental data.

$$\ln k_{alt} = a + \frac{b}{T} + \frac{c}{T^2} \quad (2)$$

Enthalpy, entropy, and heat capacity values were calculated as

$$\Delta H^\circ = -R\left(b + \frac{2c}{T}\right) \quad (3)$$

$$\Delta S^\circ = R\left(a - \frac{c}{T^2}\right) \quad (4)$$

$$\Delta C_p^\circ = \frac{2Rc}{T^2} \quad (5)$$

The Gibbs free energy was calculated as

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (6)$$

RESULTS AND DISCUSSION

Tannin Characterization. Variation in tannin structure has been found to influence interaction with various macromolecules[13−16,20,42−46] and has been shown to affect red wine

Table 1. Phloroglucinol, GPC, and PLRP-s Analytical Results for Isolated Grape and Wine Tannins

		phloroglucinolysis				GPC	PLRP-s
sample	fraction	% MC[a]	mDP[b]	% tri-OH[c]	% galloyl[d]	50% elution[e]	PAU_{520}/PAU_{280}[f]
seed	1	57.2	2.8	0	4.9	14.7	0.00
	2	68.5	3.2	0	32.1	13.4	0.00
	3	67.4	10.2	0	23.4	12.5	0.00
	4	63.4	14.4	0	23.9	12.1	0.00
	5	55.7	21.2	0	23.7	11.7	0.00
skin	1	60.0	12.5	24.2	6.2	13.1	0.00
	2	66.4	25.4	28.8	6.8	12.5	0.00
	3	71.5	39.0	38.7	5.1	11.8	0.00
	4	77.7	53.4	45.0	4.1	11.5	0.00
	5	80.7	69.4	48.3	3.6	11.2	0.00
CS2012	1	6.6	2.9	13.7	2.0	14.3	0.39
	2	15.4	6.8	17.3	1.8	13.6	0.31
	3	31.9	11.2	21.3	4.2	13.4	0.25
	4	54.3	22.7	25.1	6.5	13.0	0.15
	5	64.9	38.3	29.6	7.4	12.4	0.07
CS2010	1	18.7	2.6	15.1	1.5	13.3	0.32
	2	25.1	6.1	21.8	2.6	12.9	0.26
	3	40.5	7.4	24.2	3.7	12.6	0.17
	4	56.3	15.4	27.2	4.8	12.2	0.12
	5	62.2	24.5	34.5	3.9	11.9	0.09
CS1990	1	6.5	3.1	25.2	0.6	12.7	0.17
	2	8.0	5.0	28.3	1.0	12.7	0.16
	3	9.9	5.6	31.7	1.9	12.8	0.15
	4	12.6	9.3	37.9	2.3	12.5	0.15

[a]MC, mass conversion. [b]mDP, mean degree of polymerization. [c]% tri-OH, percent epigallocatechin. [d]% galloyl, percent (−)-epicatechin-3-*O*-gallate. [e]50% elution, 50% GPC elution time in minutes. [f]$\%A_{520/280}$, percent of absorbance at 520 nm compared with 280 nm.

sensory properties.[17,18,29,30] To provide variation in tannin structure in the present study, grape-based tannins (preveraison skin and seed) and Cabernet Sauvignon red wine tannins (2012, 2010, and 1990 vintages) were extracted, purified, and fractionated by Sephadex LH-20 chromatography, which provided subsamples varying in average molecular mass. It was desirable to separate tannin into numerous size-based fractions given the observed effect that tannin size has on tannin activity. Seed and skin tannin samples were selected because previous studies have shown that these samples differ in their astringency qualities.[17,18] The rationale for selecting the wines was that the resultant variation in tannin structure would reflect what would occur during wine production (vintage 2012 vs vintage 2010) as well as the effect of longer term aging (vintage 1990).

Phloroglucinolysis and GPC provided compositional information as well as average molecular size information. From Table 1, significant variation in average molecular size (50% elution time by GPC) and tannin composition was obtained. In addition to the effect of size, specific compositional differences that were of interest included the mass conversion of the tannin (MC) and flavan-3-ol subunit composition (tri-OH and galloyl). MC provided information on the phloroglucinolysis conversion yield of tannins into known grape subunits.[40] From previous studies, a reduction in MC has been associated with wine age and tannin oxidation.[33,47] Tri-OH and galloyl provided information on prodelphinidin content and proanthocyanidin galloylation, respectively. In addition, the incorporation of 520 nm absorbing material into the tannin polymer (PAU_{520}/PAU_{280}) provided information on the incorporation of anthocyanins into the tannin polymer, which is considered to play a role in tannin astringency modification.[48] As shown in Table 1, tannin samples varying in size and composition were prepared. It should be noted that for CS1990, very little variation in tannin size for the various fractions was observed. On the basis of the the low MC, CS1990 tannins bore little resemblance to grape-based tannins.

Retention Thermodynamics Rationale. The thermodynamics of hydrophobic interaction has been useful in understanding analyte retention in reversed-phase HPLC.[37,38] Haidacher et al., for example, were able to calculate the retention thermodynamics of various dansyl amino acid derivatives from their retention factor (k) response to column temperature.[37]

From previous work using ITC,[33] the thermodynamics of tannin hydrophobic interaction with macromolecules was found to be related to tannin structure. In the present study, to understand the hydrophobic interaction of tannins, their retention thermodynamics on a rigid spherical polystyrene divinylbenzene reversed-phase column was investigated. With this column material, and with the exception of potential intermolecular association with other tannin molecules, applied tannins would be expected to interact exclusively through hydrophobic interactions.

A separation method was developed based upon a previously published method in which tannins were separated using a

solvent gradient.[41] Using a modification of this method, purified seed tannin was evaluated at various temperatures (Figure 2B). Figure 2B shows clearly that as the column temperature increased, the peak area associated with $Tannin_P$ decreased, with a concomitant increase in the proportion of tannin eluting prior to 24 min. Conceptually, from Figure 2B, it can be seen that the retention factor for individual tannin molecules is affected by temperature. In the present case however, temperature effects on retention factor are observed as a reduction in $Tannin_P$. The purpose of this study was to develop a method that used this response to temperature for measuring the thermodynamics of interaction between tannins and the stationary phase.

To date, measuring the thermodynamics of analyte hydrophobic interaction has been limited to resolved compounds such that their retention factors can be determined. The complex nature of grape and wine tannins requires that an average retention factor that accounts for all molecular interactions is needed:

$$\frac{1}{n}\sum_{i=1}^{n} k_i \tag{7}$$

Unfortunately, and as observed in Figure 2B, the resolution of tannins in current chromatography methodologies does not allow for the measurement of the retention factors for individual tannin molecules. Because tannin activity is based upon the activity of a complex tannin system, an alternative retention factor that reflected the entire tannin mixture would be more valuable.

Given that the effect of temperature on $Tannin_P$ in this method is observed as a reduction in peak area, an alternative k (k_{alt}) was developed (eq 1). k_{alt} was observed to change as a function of temperature, and from this response, the thermodynamics of tannin retention could be measured. Using k_{alt}, the retention thermodynamics for grape and wine tannin was explored.

One potential issue with measuring the tannin interaction as a function of temperature was the observation that samples exhibited a thermochromic response, where $Tannin_T$ decreased with an increase in column temperature (ca. 10−15%). This response was consistent across all sample types (i.e., seed, skin, wine). Excluding the CS1990 samples, a plot of the peak area increase with temperature for the portion of tannin eluting prior to 24 min, against the peak area decrease for $Tannin_P$, yielded a scatter plot with a slope of 0.99, with good correlation across all samples (R^2 = 0.92). This result provided good evidence that the thermochromic response was consistent across the peaks of interest and therefore would not interfere with the determination of interaction thermodynamics. The relationship for the CS1990 samples differed, suggesting that the thermochromic response is different in older wines.

Retention Thermodynamics. The van't Hoff relationship ($\ln(k_{alt})$ vs temperature) for tannin−polystyrene divinylbenzene interaction (Table 1) was found to be nonlinear across the temperature range investigated (25−60 °C; Figure 3). This observation may be a result of tannin conformational changes that are expected to occur in this temperature range.[49] With this nonlinear response, eq 2 was fit to the van't Hoff relationship and was found to fit the observed data well (R^2 = 1.0 in all but one tannin sample).

The derived constants from eq 2 were utilized in eqs 3−5 to calculate the thermodynamic parameters (Table 2). The results

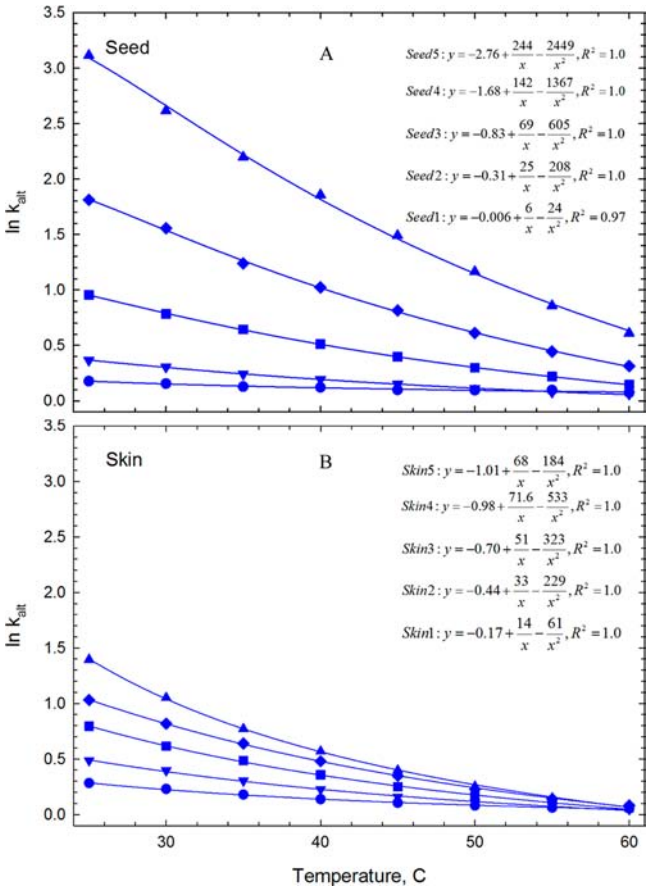

Figure 3. Relationship between the alternative retention factor ($\ln k_{alt}$) and temperature for fractionated grape seed (A) and grape skin (B) tannins.

indicated that tannin retention on the polystyrene divinylbenzene column was a spontaneous and exothermic process in all cases. The observed negative entropy of interaction in all cases is expected with solute adsorption. The thermodynamic parameters also indicated the presence of enthalpy−entropy compensation (EEC) and a consistently negative Gibb's free energy, increasingly negative with an increase in tannin size (i.e., a decrease in 50% retention time by GPC) (Table 1). EEC in reversed-phase chromatography is an indication of weak force, hydrophobic interaction.[38] The consistent EEC in the samples investigated in this study suggests a similar hydrophobic interaction across the diverse samples.

In previous studies, the molecular size of tannin has often been identified as a variable with regard to tannin activity.[25,26] This observation was investigated in the present study. Given that the tannin samples were polydisperse, the retention time at 50% by GPC was used to provide information for each sample that would be analogous to the average size of the tannin mixture. Because GPC separates samples according to their size, the assumption was made that molecular size would be related to the surface available for interaction.

The values for enthalpy and entropy of interaction for each sample source were plotted on a $\log_{10}$ scale against the 50% elution time by GPC (Figure 4). Within each sample, strong relationships were found (R^2 > 0.99), with the measured thermodynamics of interaction relating to tannin size within each sample type (e.g., seed, skin, and wine). Specifically, and normalizing for size, the enthalpy and entropy values decreased

Table 2. Thermodynamics of Grape and Wine Tannin Fraction Retention on Polystyrene Divinylbenzene Solid Support

sample	fraction	ΔH (kJ mol^{-1})	$T\Delta S$ (kJ mol^{-1})	C_p (kJ mol^{-1} K^{-1})	ΔG (kJ/mol)
seed	1	−3.631	−3.192	0.088	−0.439
	2	−10.537	−9.625	0.207	−0.911
	3	−26.022	−23.670	0.451	−2.352
	4	−46.368	−41.839	0.681	−4.529
	5	−70.959	−63.295	0.790	−7.664
skin	1	−9.318	−8.612	0.244	−0.705
	2	−16.446	−15.228	0.377	−1.218
	3	−27.200	−25.241	0.630	−1.959
	4	−33.741	−31.171	0.720	−2.570
	5	−52.279	−48.842	1.394	−3.437
CS2012	1	−1.045	−0.707	0.011	−0.338
	2	−2.862	−2.260	0.033	−0.603
	3	−3.994	−3.218	0.046	−0.776
	4	−6.334	−5.513	0.079	−0.821
	5	−17.736	−16.390	0.453	−1.346
CS2010	1	−2.316	−1.741	0.013	−0.575
	2	−4.613	−3.665	0.031	−0.947
	3	−7.399	−6.198	0.047	−1.201
	4	−12.459	−11.086	0.233	−1.373
	5	−19.370	−17.493	0.402	−1.877
CS1990	1	−5.965	−4.083	−0.039	−1.883
	2	−9.269	−6.854	0.023	−2.415
	3	−31.782	−28.459	1.006	−3.323
	4	−33.848	−29.651	0.837	−4.197

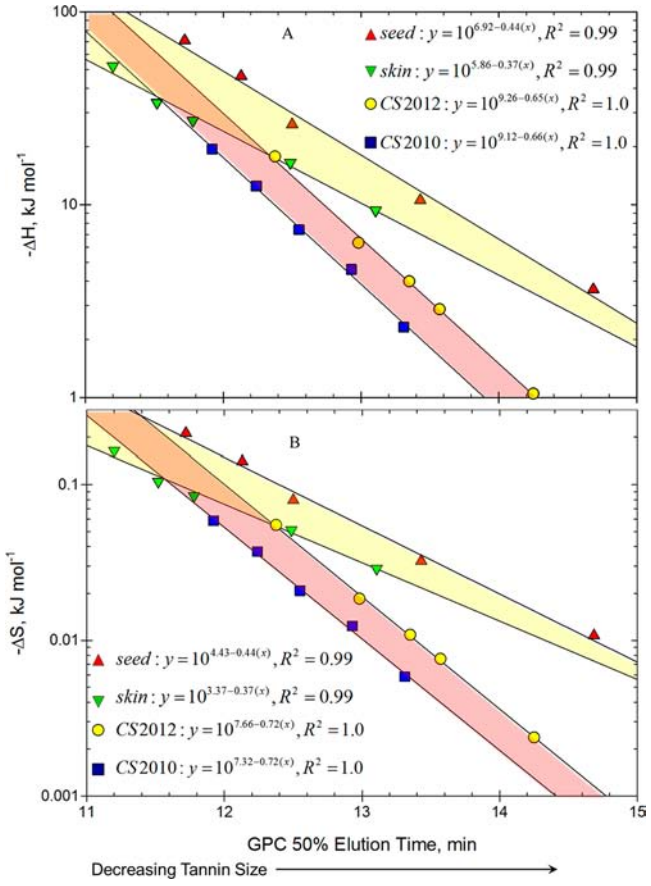

Figure 4. Enthalpy (A) and entropy (B) of interaction for grape-based (yellow) and wine-based (red) tannin interaction with polystyrene divinylbenzene versus 50% elution time by gel permeation chromatography.

in the order seed > skin > CS2012 > CS2010. Interestingly, for CS1990, there did not appear to be a relationship between tannin size and thermodynamic properties (Table 2). This may be related to the narrow range in size and composition observed for these samples.

The nonlinear relationship between thermodynamics of interaction and 50% elution time by GPC could be a reflection of the differences in tannin separation. Specifically, GPC separations are related to volume, whereas the thermodynamics are based upon surface interaction. Therefore, the axes in Figure 4 are related to molecular surface area (*y*-axis) and volume (*x*-axis). The slope change between samples in Figure 4 may be related to a change in molecular shape (thereby affecting the surface available for adsorption), or the slope change may be related to adsorption surface chemistry.

The increase in column interaction with tannin size in the present study is consistent with calorimetric measurements of tannin−protein interaction, sensory studies, and other investigations in which tannin interaction is involved (see ref 4 and references cited therein). This consistency may indicate a general process for tannin interaction with macromolecules and suggests that measuring the thermodynamics of hydrophobic interaction has importance.

On the basis of the slope of the lines in Figure 4, and for seed and skin tannins, the thermodynamics of interaction diverged as the tannin size increased (decreasing retention time), whereas for wine tannins the slopes of the lines were similar but more negative than for either skin or seed tannins. Assuming that enthalpies and entropies of interaction are related to tannin composition, these results suggest that skin and seed tannins approached a similar composition in the lower molecular weight range. On the basis of flavan-3-ol subunit composition, seed tannins varied from skin tannins in that they contain a higher proportion of (−)-epicatechin-3-*O*-gallate and lack (−)-epigallocatechin. The variation in thermodynamics of interaction between seed and skin tannins as a function of tannin size suggests that the presence of these subunits is affecting retention thermodynamics, with the thermodynamics of seed tannin interaction being more exothermic at an equivalent size.

For wine tannin samples, the incorporation of 520 nm absorbing material suggests the incorporation of anthocyanin material. The addition of anthocyanins into the tannin polymer would provide glucosylation and flavylium-containing structures, and this could explain the observed results, with a reduction in 520 nm material noted as the tannin size increased, and eventually resembling the thermodynamics of skin tannin interaction.

In a comparison of the compositional information of CS2012 and CS2010 samples (Table 1) with their respective thermodynamics of interaction (Figure 4), there does not initially appear to be an immediate explanation for the observed differences for these samples. Upon closer examination, the relationship between the average degree of polymerization (mDP) by phloroglucinolysis was different from the elution

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time at 50% by GPC, with CS2012 samples having a higher mDP at an equivalent elution time. Added to this, CS2012 had a reduced 520 nm absorbance at an equivalent size. Taken together and normalizing for tannin size, these results suggest that younger wine tannins have a reduced incorporation of anthocyanins and are consistent with anthocyanin incorporation reducing tannin interaction with macromolecules.

In addition to enthalpy and entropy of interaction, the heat capacity values for the tannins were also plotted against 50% elution time by GPC (Figure 5). The heat capacity increased

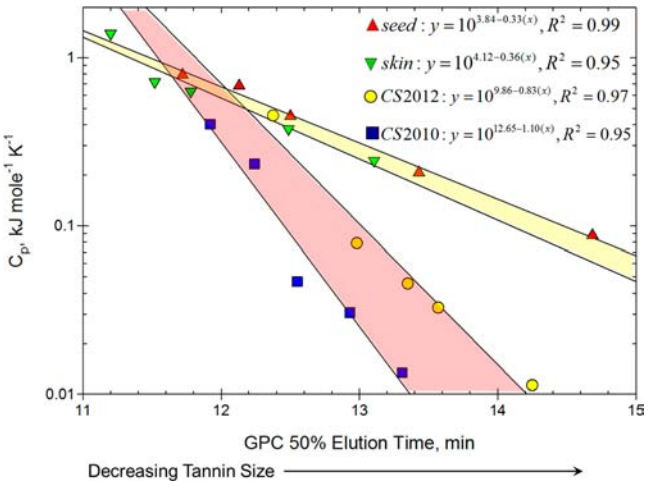

Figure 5. Heat capacity values calculated from interaction of grape-based (yellow) and wine-based (red) tannins with polystyrene divinylbenzene versus 50% elution time by gel permeation chromatography.

with tannin size for all samples. This increase can be interpreted as an increase in molecular degrees of freedom with size. When normalized for size, seed and skin tannins had similar heat capacities and are structurally similar. The addition of galloylation may explain the increase in heat capacity between seed and skin tannins. Albeit speculative, the decline in heat capacity for wine tannins may be related to a general decline in mass conversion. A reduction in mass conversion may be related to tannin oxidation and the formation of additional intramolecular interflavonoid bonds leading to restricted rotation.[12]

The goal of this project was to develop new analytical approaches to understand how astringency in grapes and wine changes in transition from fruit to new wine and finally to older wine. These stages are important control points that are managed during grape and wine production yet currently lack adequate analytical methodology for effective management. Whereas the anecdotal assumption has been that tannin structure is important to red wine astringency quality, a routine method for measuring structure−activity relationships has been elusive. With the general assumption that tannin association with proteins and, in this case, polystyrene divinylbenzene involves hydrophobic interactions, this HPLC method provides an avenue for improving our understanding of red wine astringency quality. The general observation from this paper is that the thermodynamics of interaction for complex systems can be obtained using HPLC, and this result may have applications beyond tannin chemistry. In studies in which the thermodynamic activity of complex macromolecules from biological and synthetic sources is of interest, this analytical approach may find application.

AUTHOR INFORMATION

Corresponding Author

*Phone: +1 (559) 278-7179. Fax: +1 (559) 278-4795. E-mail: jakennedy@csufresno.edu.

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