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# Thermometric Titration of Polyhydric Phenols. Application to the Determination of the Tannin Content of Wines

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Various oxidants for the thermometric titration and back-titration of polyhydric phenols were studied. The results obtained with polyhydric phenol standards consisting of gallic or tannic acid showed potassium hexacyanoferrate(III), cerium(IV) sulfate and potassium permanganate to be the most suitable oxidants for the purpose. The use of excess potassium permanganate or cerium(IV) for subsequent back-titration with Mohr's salt was ruled out on the grounds of kinetic considerations. Potassium hexacyanoferrate(III) and potassium permanganate (first equivalence point) provided consistent results; however, the former gave rise to sharper equivalence points in titrations involving increased polyhydric phenol concentrations. Titrations of tannins present in wine with potassium hexacyanoferrate(III) or cerium(IV) provided results that were also consistent with those obtained by using the classical Löwenthal volumetric method and the Folin-Ciocalteu method. Thermometric titrations with potassium hexacyanoferrate(III) or cerium(IV) have the added advantage that, unlike the Löwenthal method, they do not require oxidation restrictors or matrix correction. In addition, the presence of sulfur dioxide or reducing sugars in the wine interferes much less severely with the thermometric titration than it does with the Folin-Ciocalteu method. If ascorbic acid were present it would interfere.

**Keywords:** Tannin; polyhydric phenol; thermometric titration; wine

Tannins are polyhydric phenol compounds occurring in plants and fermented beverages. Their role in preserving the anthocyanine in wines and juices, where they are used as clarifying agents, is of interest. They have a reducing character that has been widely used for their determination.<sup>1,2</sup> Some common oxidants allow tannins to be determined by straightforward titrimetry, whereas others give rise to coloured substances and can be determined spectrophotometrically (e.g., with the Folin-Ciocalteu<sup>3</sup> or Prussian Blue method). Spectrophotometric methods can be used not only for determining polyhydric phenols, but also for detecting them chromatographically.

Tannins undergo complex redox reactions, many of which are slow or very slow. This is the most serious drawback to be faced in their volumetric titration as they require very low rates of reagent addition and some heating to favour reaction development. Fast reactions would lead to increased selectivity in titrations of tannins, as slower reductants potentially present in real samples (e.g., proteins, reducing sugars) are bound to interfere much less seriously, if at all.

The Löwenthal method,<sup>1,2</sup> which involves the titration of polyhydric phenols with permanganate ion, uses an indicator (Indigo Carmine) to exclude all those substances that react more slowly than itself. Attempts at monitoring Löwenthal titrations by measuring the redox potential of the reaction medium have so far met with no success.<sup>4</sup> Alternative usable oxidants include potassium hexacyanoferrate(III) and cerium(IV) sulfate.<sup>1,2,4-6</sup> However, no restrictors appear to have been used for the oxidation of tannins.

Thermometric titrations represent a useful alternative approach to the titration of tannins. They allow one to distinguish between two or more reactions or groups of reactions based on successive changes in the slope of the recorded thermometric curve. In addition, this avoids one other shortcoming of traditional volumetric determinations for tannins, *viz.*, the difficulty of detecting the equivalence point arising from the various colours a given sample might have before and after tannins have been oxidized.

Notwithstanding the potential usefulness of this technique, no references have been found in the literature to the determination of tannins, although Priestley<sup>7</sup> used the highly exothermic reaction of cerium(IV) in the oxidation of polyhydric phenols for the calibration of a digital titrator. The aim of this work was to test the classical oxidizing titrants of

tannins (and a few others not explicitly cited in the literature) in the thermometric titrimetric determination of tannins. The Folin-Ciocalteu reagent was also tested.<sup>3</sup>

## Experimental

### Apparatus

The thermometric set-up used was identical with that reported by Morante *et al.*<sup>8</sup> and consisted of a Metrohm Dosimat E501 autoburette, an adiabatic thermometric cell equipped with a fast-response, thermometer-type thermistor (nominal resistance 100 kΩ at 25 °C), a Wheatstone bridge for measuring its resistance and an *x-t* recorder.

All experiments were carried out at 23 ± 1 °C in a thermostated laboratory room.

### Reagents

The reagents used included 5 mol l<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>, 3 mol l<sup>-1</sup> NaOH, 1 g l<sup>-1</sup> gallic acid (Fluka), 1 g l<sup>-1</sup> tannic acid (Fluka), 0.1, 0.06 and 0.02 mol l<sup>-1</sup> potassium permanganate (standardized with sodium oxalate), 0.1 mol l<sup>-1</sup> Ce(SO<sub>4</sub>)<sub>2</sub> in 0.5 mol l<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> (also standardized with sodium oxalate), 0.1 mol l<sup>-1</sup> potassium hexacyanoferrate(III) (prepared from the recrystallized analytical-reagent grade compound) and 0.1 mol l<sup>-1</sup> ammonium iron(II) sulfate (Mohr's salt) in 1 mol l<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> standardized with Ce(SO<sub>4</sub>)<sub>2</sub>.

### Procedures

All thermometric titrations were performed in a cell containing the standard or sample to be titrated, some acid or base to adjust the pH, the reagents specifically required and distilled water to 50 ml. The titrant was added from the autoburette to the analyte in the cell, which was maintained under vigorous shaking. The over-all volume added was always less than 5 ml. The recorder sensitivity was set in accordance with the heat produced in the reactions.

#### Direct thermometric titrations

The procedure followed in each instance depended on the oxidant used.

**Titrations with  $KMnO_4$ .** Gallic acid concentrations between 50 and 300 mg l<sup>-1</sup> in the cell were assayed. The reaction medium was made 1 mol l<sup>-1</sup> in  $H_2SO_4$ . The  $MnO_4^-$  concentration used varied between 0.1 and 0.02 mol l<sup>-1</sup>, and its rate of addition was 2.5 ml min<sup>-1</sup> for the former concentration and 1 ml min<sup>-1</sup> for 0.1 and 0.06 mol l<sup>-1</sup>. The sensitivity was 0.5 °C on the background scale.

**Titrations with  $Ce(SO_4)_2$ .** The standard concentrations used were the same as in the previous procedure. The  $H_2SO_4$  concentration used was 0.5 mol l<sup>-1</sup>. The titrant, 0.1 mol l<sup>-1</sup>  $Ce(SO_4)_2$  solution, was added at a rate of 2.5 ml min<sup>-1</sup>. The sensitivity was 0.2 °C on the background scale.

**Titrations with  $K_3Fe(CN)_6$ .** The gallic (and tannic) acid concentrations used varied from 50 to 200 mg l<sup>-1</sup>. Titrations were carried out in a basic medium (0.3 mol l<sup>-1</sup> NaOH) by using a titrant concentration of 0.1 mol l<sup>-1</sup> at a rate of addition of 2.5 ml min<sup>-1</sup>. The sensitivity was 0.2 °C on the background scale.

#### Thermometric back-titrations

All such titrations were performed in 1 mol l<sup>-1</sup>  $H_2SO_4$ . Over the gallic acid in the titration cell was added an excess of oxidant, which, after 1 h ( $Ce^{IV}$ ) or 30 min ( $MnO_4^-$ ), was titrated thermometrically with 0.1 mol l<sup>-1</sup> Mohr's salt.

Both cerium(IV) and potassium permanganate were used as titrants in these back-titrations. Their rate of addition was 2.5 ml min<sup>-1</sup> and the sensitivity was 0.2 °C on the background scale.

## Results and Discussion

The following oxidants were studied in order to determine whether they produced measurable temperature changes on reaction with the polyhydric phenols: potassium bromate, potassium permanganate, potassium iodate, potassium hexacyanoferrate(III), ammonium vanadate, cerium(IV) sulfate, potassium peroxodisulfate and potassium dichromate. One other requirement was that the measured parameter value at the equivalence point should be proportional to the amount of standard or sample in the cell. Preliminary tests involved using the oxidants on gallic acid, a standard widely employed among others (tannic acid, etc.) for determining tannins.<sup>9</sup>

Potassium bromate, which was used in HCl and  $H_2SO_4$  media, provided thermometric curves with equivalence points that were not proportional to the polyhydric phenol concentration. This also occurred with potassium iodate, despite the fact that it yielded a seemingly usable response. As regards ammonium vanadate and potassium dichromate, the volume of oxidant added did not increase proportionally to the amount of substrate.

Potassium peroxodisulfate gave rise to a gentle slope change of the thermometric curve. Also, the recording obtained with the Folin-Ciocalteu reagent was indistinguishable from that of the blank as the heat of dilution produced masked that arising from any concurrent oxidation reaction.

The only potentially useful oxidants that remained were potassium permanganate, cerium(IV) sulfate and potassium hexacyanoferrate(III), i.e., those typically used in classical titrations of polyhydric phenols. These were the only ones that met the above requirements on addition to gallic acid. Fig. 1 shows typical curves obtained with each of these three reagents. Also, as can be seen from Fig. 2, the amount of oxidant used was proportional to that of gallic acid present in the reaction vessel.

### $KMnO_4$ Titrant

Provided that it was used at a concentration above 0.3 mol l<sup>-1</sup>,  $KMnO_4$  gave rise to two equivalence points (Fig. 1, line A), of which the first was much sharper than the second. The former

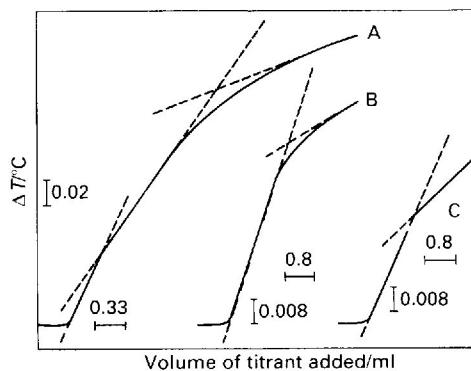


Fig. 1 Thermometric curves obtained in the titration of 100 mg l<sup>-1</sup> of gallic acid with various oxidants: A, 0.3 mol l<sup>-1</sup>  $KMnO_4$ ; B, 0.1 mol l<sup>-1</sup>  $K_3Fe(CN)_6$ ; and C, 0.1 mol l<sup>-1</sup>  $Ce(SO_4)_2$

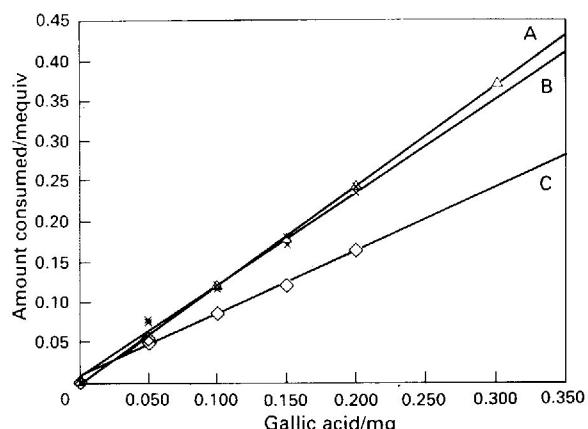


Fig. 2 Linear relationship between the amount of standard in the titration cell and the amount of oxidant used in the thermometric titration. Titration with: A,  $KMnO_4$ ; B,  $K_3Fe(CN)_6$ ; and C,  $Ce(SO_4)_2$

can be ascribed to the faster and the second to the slower reactions.

The first equivalence point was reached by adding about 5 equiv ( $4.7 \pm 0.2$ ,  $n = 7$ ) of  $MnO_4^-$  per mole of gallic acid, whereas the second required about 11 equiv ( $11.1 \pm 1.2$ ,  $n = 5$ ). It was decided to use the first equivalence point on the grounds of its greater sharpness. However, if the gallic acid concentration in the cell was high enough, such a point was ill-defined or even undetectable; this cannot be exclusively ascribed to thermodynamic considerations, which supports the above kinetic interpretation. It was finally decided to use 0.02 mol l<sup>-1</sup>  $MnO_4^-$ , which provided a wider working range, even though it only allowed one to observe the first equivalence point. Even so, the sharpness of such a point decreased with increase in the polyhydric phenol concentration.

### $K_3Fe(CN)_6$ Titrant

The amount of  $K_3Fe(CN)_6$  needed to oxidize 1 mol of gallic acid was  $4.5 \pm 0.1$  equiv ( $n = 12$ ), i.e., virtually the same as for  $MnO_4^-$ ; however, the titration curves were sharper over a wider concentration range than those provided by the latter (Fig. 1, line B).

For comparison, gallic acid was also titrated with permanganate in the classical manner. As in the Löwenthal method, Indigo Carmine was used as oxidation limiter. This should have provided results comparable to those of the direct thermometric titrations. After the amount of titrant used by the indicator was subtracted, 1 mol of gallic acid was found to require  $4.5 \pm 0.1$ ,  $n = 3$  of oxidant.

**Ce<sup>IV</sup> Titrant**

An amount of about 4 equiv ( $3.7 \pm 0.1$ ,  $n = 8$ ) of cerium(IV) was required to oxidize 1 mol of gallic acid. This is inconsistent with the results obtained for the other oxidants examined. However, the recorded curves had sharper equivalence points (see Fig. 1, line C).

**Back-titration**

Back-titration were conducted in order to check the assumption that the oxidation process involved many more reactions than those taken into account in direct thermometric titrations, reactions of various origins proceeding at widely different kinetic rate constants. In addition, they may provide useful alternatives to direct titrations as they yield sharper equivalence points. This is so provided that an appropriate reductant (e.g., Mohr's salt) is used to back-titrate the excess oxidant. The chief shortcoming of this procedure lies in the need to allow the reaction to proceed to completion before the titration proper is started in order that reproducible results can be obtained. The back-titration with permanganate proved to be relatively reproducible as the reaction time (about 30 min) was long enough for the reaction to be completed or to proceed so slowly as to prevent small differences in the analysis time from affecting the results significantly.

Complete oxidation of gallic acid required  $16.3 \pm 0.4$  equiv ( $n = 12$ ) per mole. The reaction of cerium(IV) with tannins is believed to be slower than that of  $MnO_4^-$ .<sup>5</sup> In order to check this assumption, the standard was brought into contact with  $Ce(SO_4)_2$  in the adiabatic cell for different lengths of time before the excess was back-titrated with Mohr's salt. Two reaction cells were used for each point in time considered. Fig. 3 shows the milliequivalents of cerium(IV) consumed per gram of two standards (gallic and tannic acid) as a function of the reaction time elapsed before the beginning of the thermometric back-titration of the excess, with iron(II). The reaction time was expected to be long on account of the concentrations and temperature used. However, reaction for 1 h was sufficient to avoid the effect of small variations in the analysis time on the titration results. As expected, the number of equivalents of gallic acid used in the back-titration was much greater than that used in the direct titrations. In fact, it was typically 47 mequiv  $g^{-1}$  ( $8.9 \pm 0.4$  equiv  $mol^{-1}$ ,  $n = 7$ ) for gallic acid and about 30 mequiv  $g^{-1}$  for tannic acid.

Back-titration do not allow the faster reactions to be distinguished from the slower reactions. Therefore, if real samples are to be assayed, then slowly reacting reductants (e.g., proteins and reducing sugars) should be previously determined by using one of the recommended procedures.<sup>5</sup> Hence back-titration methods cannot be used if a rapid analysis is needed.

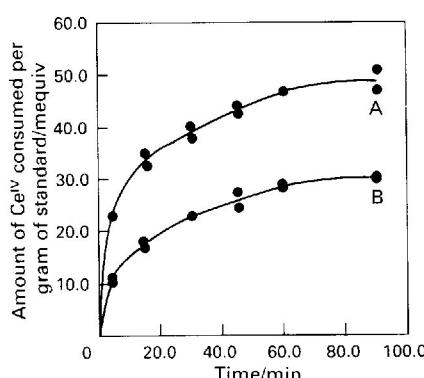


Fig. 3 Time course of the oxidation of two polyhydric phenol standards with  $0.1\text{ mol l}^{-1} Ce(SO_4)_2$ . A,  $100\text{ mg l}^{-1}$  gallic acid; and B,  $200\text{ mg l}^{-1}$  tannic acid

**Applications**

By way of example, the thermometric procedure was applied to the determination of polyhydric phenols in wines by using  $K_3Fe(CN)_6$  and cerium(IV) as oxidants. For contrast, we also employed the Löwenthal method as modified for wines, which uses Indigo Carmine as oxidation restrictor. A synthetic wine was used for the evaluation of the non-tannin reducing background (ethanol, reducing carboxylic acids).<sup>10</sup> The results were also compared with those provided by a spectrophotometric method for the determination of tannins using the Folin-Ciocalteu reagent, the automated version of which was developed in our laboratory.<sup>9</sup>

All wine samples were appropriately diluted, between 1 + 4 and 1 + 9 for red wine and between 1 + 1 and 1 + 2 for white wine.

The results for tannins are typically delivered in one of the following forms. (i) Relative to the type of measurement,<sup>10</sup> in milliequivalents used per litre of sample (e.g., the permanganate number) or, with spectrophotometric methods, the absorbance obtained under pre-set conditions (Folin-Ciocalteu method).<sup>10</sup> This format was used for the data listed in Table 1, which only allow for comparison with those provided by methods based on redox titrations. (ii) Relative to a polyhydric phenol standard (usually gallic or tannic acid) on the assumption that the standard reacts similarly to the unknown sample in a given range of experimental conditions. Even though the accuracy of this procedure is arguable, this is the only way in which the results provided by methods based on different techniques can be compared (Table 2).

As can be seen from Tables 1 and 2, the results provided by all the methods were similar. The results are more variable in Table 2 as gallic acid reacts in a different way in the presence of the different oxidants. The results in Table 1 are more concordant. The sample tannin gave better agreement between methods, possibly because a mixture of substances is present, making up their different reactivity. This behaviour has been described previously.<sup>4</sup>

Table 1 Results obtained in the analysis of wine samples by using two thermometric methods and a reference method

Sample	Oxidant consumed/mequiv $l^{-1}$		
	Method 1*	Method 2†	Reference method‡
White wine	$3.9 \pm 0.3$	$3.8 \pm 0.2$	$4.1 \pm 0.3$
Rosé wine	$7.8 \pm 0.1$	—	$8.0 \pm 0.3$
Red wine	$21.1 \pm 1.5$	$17.7 \pm 1.6$	$21.4 \pm 0.3$

\* Direct titration with hexacyanoferrate(III).

† Direct titration with  $Ce^{IV}$ .

‡ Löwenthal method.

Table 2 Results obtained in the analysis of wines by using two thermometric methods and two reference methods

Sample	Gallic acid/ $mg l^{-1}$			
	Method 1*	Method 2†	Reference method 1‡	Reference method 2§
White wine	137	192	174	114
Rosé wine	271	—	283	298
Red wine	778	857	860	746

\* Direct titration with hexacyanoferrate(III).

† Direct titration with  $Ce^{IV}$ .

‡ Löwenthal method.

§ Folin-Ciocalteu method.

### Interferences

Some reductants typically present in wines (sulfur dioxide, ethanol, glucose and ascorbic acid) are potential interferents with the determination of tannins by oxidation.<sup>9</sup> The effects of these substances were investigated by using white wine matrices and gallic acid standards with tannin contents similar to those typically found in white wines. This type of wine was chosen on the grounds of its low tannin content, which would enhance the potential effect of the interferents.

The maximum permitted concentration of sulfur dioxide in wines is 200 mg l<sup>-1</sup>. A concentration of 200 mg l<sup>-1</sup> in the reaction cell (*i.e.*, the equivalent of 400 mg l<sup>-1</sup> in a white wine diluted 1 + 1) consumed no titrant and resulted in no increase in the amount of hexacyanoferrate(III) used to titrate gallic acid or wine spiked with sulfur dioxide.

Likewise, a 5% ethanol content in the cell (*i.e.*, 10% in white wine diluted 1 + 1) consumed no oxidant, whether alone or in the presence of gallic acid.

As regards reducing sugars, a concentration of 8 g l<sup>-1</sup> of glucose in the cell had no effect on the titration. On the other hand, an ascorbic acid concentration as low as 5 mg l<sup>-1</sup> used a significant amount of hexacyanoferrate(III), which added up to that consumed by gallic acid when titrated together, or to that of wine spiked with this acid. Consequently, the analysis of white wine is interfered with by ascorbic acid at concentrations as low as 10 mg l<sup>-1</sup>. Ascorbic acid has been used in wines owing to its reducing capacity.<sup>10</sup> The maximum tolerated concentration according to Spanish legislation is 100 mg l<sup>-1</sup>. Its presence permits the amount of sulfur dioxide to be decreased and the wine to be protected against the 'casse ferrique'.

### Conclusions

The results obtained in this work show the feasibility of determining tannins in wines by direct thermometric titration with potassium hexacyanoferrate(III) or cerium(IV) sulfate. Neither of the two oxidants present clear advantages. Cerium(IV) titrations give sharper end-points, but potassium hexacyanoferrate(III) offers results closer to those of Löwenthal's method. In both instances the results were fairly

good and comparable to those provided by the well known Löwenthal method, but with no matrix correction being required, and to those yielded by the Folin-Ciocalteu spectrophotometric method.

In addition, the titration with potassium hexacyanoferrate(III) is free from some major interferences that affect classical methods. In fact, the only serious constraint in this respect is the low tolerance to ascorbic acid, present in wines as an antioxidant.

All methods based on redox reactions are non-specific. However, they are useful for the food scientist, as they correlate well with the degree of astringency as determined organoleptically.<sup>1</sup>

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