UV Spectrophotometric Method for Polyphenolic Tannin Analysis

M.-L. Antoine, C. Simon, A. Pizzi

ENSTIB-LERMAB, University of Nancy 1, Epinal, France

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ABSTRACT: A UV spectrophotometric method for the determination of the total phenolic content of both polyflavonoid and hydrolyzable tannins was presented. The method was compared with the standard method used today for the determination of tanning material in tanning extracts for leather manufacture. In narrow concentration ranges correlation between optical density and phenolic concentration was linear. In wider concentration ranges exponential correlations satisfactorily fitted the experimental data. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 91: 2729–2732, 2004

Key words: UV spectrophotometry; phenolic content; analysis method; hydrolyzable tannins, condensed tannins

INTRODUCTION

Polyphenolic tannins of the hydrolyzable and condensed types traditionally have been analyzed on the basis of their tanning power and thus on their capability to tan hides to produce leather. For more than a century the classic test for this purpose has been the hide powder method,[1–3] which consisted of titrating dried hide powder produced under a set of standard conditions, and of set composition, with a tannin extract solution. The method worked well but has now been discontinued and superseded by a more modern titration method, the Divergan method (based on tannin precipitation by absorption on polyvinylpyrrolidone). Both these methods, however, although well adapted to the determination of the tanning power of the material, have a considerable defect with respect to the determination of the total phenolic material of a tannin extract. Flavonoid and hydrolyzable tannins are composed of phenolic monomers and oligomers. The two titration methods above are not able to determine the presence of either phenolic monomers or dimers in the tannin extract because these do not tan hides in leather production. This is perfectly acceptable when the tannin extract needs to be analyzed for leather-making, but unacceptable when its phenolic content is needed for other uses.

Today tannins are experiencing a multitude of relatively new uses, such as wood adhesives and other resins,[4,5] food and cellular antioxidants,[6–8] and a multitude of other medical and pharmaceutical applications such as antitumor,[9,10] antiviral,[11] and antiinflammatory[12] applications, all relying on their total phenolic content. For these the total phenolic content of the tannin extract must be known, and not just its polyphenolic tanning component. Furthermore, the method of analysis chosen for this needs to be simple and use simple routine equipment that can be found in most laboratories. The only method that satisfies these requirements is a dated ultraviolet (UV) spectrophotometry method that was developed as far back as 1951, exclusively for mimosa polyflavonoid tannins,[13,14] and which is not in present use. This method was tried only for polyflavonoid condensed tannins, more precisely only for mimosa bark tannin extract, and in a very narrow range of concentrations. To be useful today it must be upgraded to a wider range of tannin concentrations and extended to hydrolyzable tannins for which it was never tried or developed.

This article focuses on extending the UV spectrophotometric analysis method to hydrolyzable tannins and to upgrade it for use with a wider set of concentrations also for polyflavonoid condensed tannins.

EXPERIMENTAL

UV method

The UV method is based on principles already published in 1951,[13] and it is based on the measure of the optical density of the benzene chromophore groups of phenolic and tannin solutions at 280 nm. At this frequency UV spectra show a well-defined peak. Here strictly only the method used is reported. Industrial tannin extract powders of chestnut (*Castanea sativa*) wood extract (ex Silva, Italy) and of mimosa (*Acacia mearnsii*, formerly *mollissima*, de Wildt) bark tannin extract (ex Tanac, Brazil) were used. Three solutions were prepared as follows:

Correspondence to: A. Pizzi.

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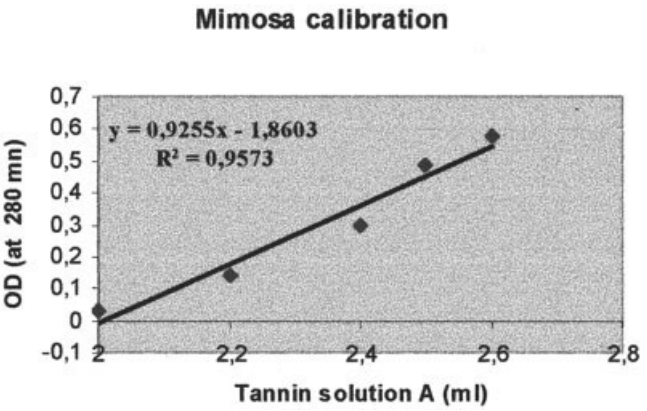

Figure 1 Linear regression analysis of the UV optical density (OD) at 280 nm of water solutions of polyflavonoid mimosa tannin extract as a function of tannin concentration in a narrower range of concentrations.

1. Zero-point standard: 0.042 g benzoic acid in 100 mL distilled water. This solution was then diluted, 2 mL solution to 10 mL water.
2. Base solution of tannin (solution A): a 1 g sample of industrial chestnut or mimosa tannin extract of known composition was dissolved by bringing it to 1000 mL volume with distilled water in a glass measuring flask.
3. A 0.2% solution of sodium bisulfite: sodium bisulfite (0.2 g) was dissolved and brought up to 100 mL volume with distilled water in a glass measuring flask. This solution is necessary to stabilize the tannin solutions to atmospheric oxidation.

The calibration curve was then determined as follows: between 2 and 2.8 mL of the base solution (solution A) of tannin was diluted with 50 mL of the 0.2% sodium bisulfite solution plus 50 mL distilled water. These were placed in a quartz cell (1 cm thick) against an equivalent cell containing the zero-pint standard solution. The measures of optical density (OD) were carried out in absorbance at 280 nm on a Hitachi U-2001 UV spectrophotometer (Hitachi, Ibaraki, Japan).

The concentration in phenolic materials of solutions of chestnut wood tannin extract at different stations in the production line of a tannin factory (Nuova Rivart, Siena, Italy) were also analyzed. Comparison was made of the results obtained with the above UV method and the calibration curve with the tanning content analysis by the Divergan polyvinyl pyrrolidone method (commonly known in the tannin trade as the Divergan method). This method of analysis is as follows.

Polyvinylpyrrolidone (Divergan) method

Approximately 6 g of dry tannin extract (the exact weight was measured on an analytical balance) were

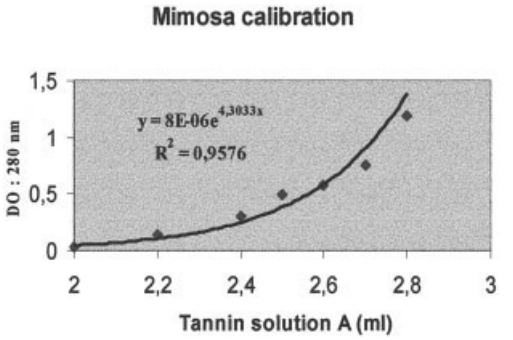

Figure 2 Nonlinear regression analysis of the UV optical density (OD) at 280 nm of water solutions of polyflavonoid mimosa tannin extract as a function of tannin concentration in a larger range of concentrations.

dissolved in distilled water at 60–70°C, brought up to volume in a 500-mL glass measuring flask, after which the solution was cooled to about 20°C (solution A).

To determine the percentage total solids (%TS) content, 25 mL of this solution were evaporated in a preweighed (on an analytical scale) stainless-steel or glass open round-bottom capsule partially immersed in a water bath at 100°C. After all the solution evaporated, the solid residue and the capsule after cooling in a dessicator were weighed and the %TS content of the solution was determined. The moisture content of the original powder extract was then determined as moisture content (%) = 100 − %TS.

If solution A was not clear it was then necessary to determine the soluble solids (SS). If it was clear the total solids and soluble solids coincided. If solution A was turbid then after filtration on a 0.45-μm membrane the same procedure used for TS was repeated starting from the filtered solution.

The nontannins (NT) were then determined. A volume lower than 50 mL of solution A was charged and,

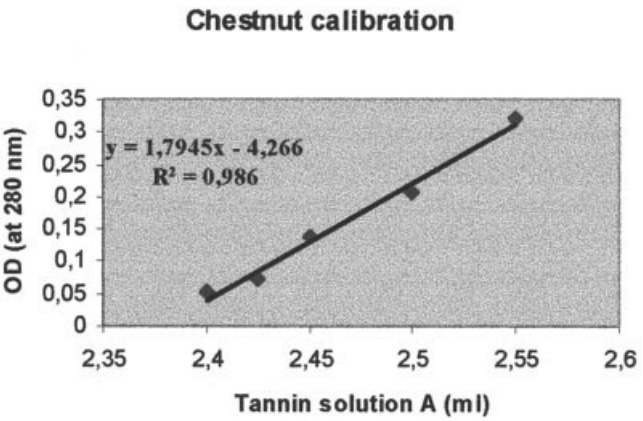

Figure 3 Linear regression analysis of the UV optical density (OD) at 280 nm of water solutions of hydrolyzable chestnut tannin extract as a function of tannin concentration in a narrower range of concentrations.

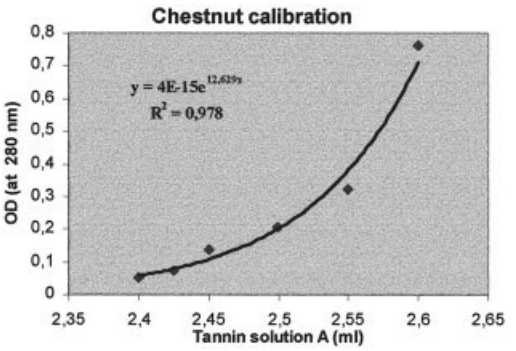

Figure 4 Nonlinear regression analysis of the UV optical density (OD) at 280 nm of water solutions of hydrolyzable chestnut tannin extract as a function of tannin concentration in a larger range of concentrations.

after addition of 1 mL 88% formic acid, brought to volume with distilled water to a 100-mL glass measuring flask (solution B). Divergan HM (7 g; Merck, Darmstadt, Germany) was charged to a filter column (27 mm diameter), equipped with a filtering membrane of porous polyethylene. A 50-mL aliquot of solution B was then filtered under a constant water vacuum pump depression of 200 mmHg, after which 30 mL of filtrate was collected. A 25-mL sample of this filtrate was evaporated in a preweighed (on an analytical scale) stainless-steel or glass open round-bottom capsule partially immersed in a water bath at 100°C. Once dry the capsule was placed in an oven at 100°C for 4 h, then cooled for about 15 min in a dessicator and weighed on an analytical balance. The percentage NT was then determined by the ratio (residual weight/original weight) × 100. The percentage tanning materials was determined then as Tannin (%) = %SS − %NT. A Divergan blank was needed for each Divergan batch. The blank was performed by substituting with 50 mL distilled water the 50 mL of solution A in the NT percentage determination procedure.

RESULTS AND DISCUSSION

For mimosa polyflavonoid tannin extract the method was followed as published in 1951.[13] However, some differences of results were observed. In the case in which only relatively narrow ranges of concentration (still wider than that used in the original method) were used, the correlation between optical density (OD) and solution concentration (and thus percentage polyphenolics content of the extract) was indeed linear, as originally reported. This result is shown in Figure 1, where the relevant linear regression equation (R^2 = 0.957) is also reported. However, when wider concentration ranges for the tannin extract were examined, the correlation followed an exponential law, reported in Figure 2, with coefficient of correlation R^2 = 0.958. A linear correlation in this case is very poor, as can be appreciated from the results in Figure 2, and deviations from linearity start to occur over 1.1 of OD.

In the more unusual case of the chestnut wood tannin extract, a hydrolyzable tannin,[15] for which this analysis method was never used before, similar but even slightly more marked trends can be observed in Figures 3 and 4. Thus, the linear fits in narrower concentration ranges also respond well to linear regression analysis (Fig. 3, R^2 = 0.986). However, when the concentration range starts to widen, mainly when the maximum concentration of the extract diverges from the range in Figure 3, the relationship between concentration and OD, although very good, cannot be described by a linear relationship. In Figure 4 this can be seen with an exponential curve fit having a coefficient of correlation R^2 = 0.978.

The results in Figures 2 and 4 indicate that the method (i) is simple to use; (ii) is adequate for the analysis of the phenolic content of all types of tannins, both polyflavonoid and hydrolyzable types; and (iii) is reliable over a relatively wider range of concentrations than originally thought if nonlinear regressions are used for the calibration curves.

Finally, the method was used to analyze the concentration in phenolic materials of solutions of chestnut wood tannin extract at different stations in the production line of a tannin factory. For comparison the samples were tested both by the Divergan method, yielding the actual tanning polyphenolic content, and by the UV method, yielding the total phenolic material of the extract. The results, presented in Table I, show that the

TABLE I
Factory Trials Results of the Two Methods for Chestnut Hydrolyzable Tannin

Sample no.	Total solids content (%)	Concentration (%)		UV method: total phenolic content (%)	Tannin and phenolics on dry extract by	
		Tannins	Nontannins		Divergan (%)	UV (%)
1	14.91	10.82	4.09	13.12	72.57	87.99
2	14.04	11.38	2.65	13.12	81.05	93.45
3	12.87	9.94	2.92	11.58	77.23	89.98
4	43.98	36.8	7.08	42.79	83.67	97.29

Divergan method always yields a consistently lower percentage value, as expected, than does the UV method.

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