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Tannins: Major Sources, Properties and Applications

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ABSTRACT

After a brief historical introduction and the distinction between hydrolysable and condensed tannins, a description of their chemistry and a short historical review on their use in leather tanning, the more recent developments in tannins for adhesives with and without the use of any aldehyde-yielding compounds, even without the use of any hardeners, are described. Examples of the use of tannins for other industrial, nonleather, applications are reported. In particular, this chapter focuses briefly on their new intended use in the medical and pharmaceutical fields. New data on their antiviral effectiveness against a great number of different viruses compared to their higher, lower or absent cytotoxicity are also presented.

Keywords

Tannins, History, Sources, Adhesives, Wood, Resins, Antiviral activity, Cytotoxicity, Leather, Nonleather uses, Pharmaceutical, Medical

8.1 HISTORY OF TANNINS EXTRACTION

Tannins are a renewable resource that is 'coming of age' in several fields different from their usual, classical application, namely hide tanning to produce heavy duty leather. Leather tanning has been used for centuries, millennia in fact, by immersing hides in pits in which tree bark or wood rich in tannin, such as oak, had been left in. This method took up to one full year to produce good leather. However, the actual tannin extraction industry is relatively more recent. It started in Lyon, France and in northern Italy in the 1850s to satisfy the need for black dyes for silk clothes. As the fashion of women silk blouses waned towards the 1870s, the multitude of small chestnut tannin extraction factories that had sprung up underwent a dramatic change of fortune, many going into bankruptcy and closing down, others combining to build enough critical mass to find an alternative use for tannin extract [1].

The few surviving producers, managed to convince the leather manufacturers that, by dissolving tannin extract in the treatment pits, leather could be manufactured in just 1 month, instead of the 1 year it took on an average with the traditional bark bath technology. As a consequence, the tannin extraction industry started its second life for an application quite different from the original one. The advantages of tannin extract in leather making, and the timesaving involved by its use, were such that the industry underwent rapid expansion and prospered. The short availability of materials in Europe to satisfy the rapidly growing demand for tannin extracts for leather prompted the opening of factories in far-away countries and the use of new types of tannins. Thus, in the early 1900s, tannin factories using quebracho tannin from South America and Mimosa tannin from Southern and Central Africa started their extraction in industrial quantities and exported them to the main northern hemisphere markets.

The two World Wars gave a considerable impulse to the expansion of tannin extraction, considering that all the armies marched on shoes with leather soles. To give a typical quantitative example, in 1946, just after the end of World War II, a major producer such as South Africa manufactured about 110 000 metric tons of dry tannin extract solids. That year however, was the zenith of the use of vegetable tannins for leather making, because from then on rubber and neoprene soles in everyday shoes, cheaper and readily available, progressively replaced leather counterparts. By the beginning of the 1970s, the total tanning production was down to 72 000 tons per year. This was followed by a further decrease in a period in which hides supply dwindled, due to the decrease and difficulties of cattle farming in the 1960s and 1970s. Finally, the considerable shift in customers' tastes in shoes, which came about with the comfortable sport and leisure shoes 'boom', gave a final blow to the use of tannins in leather making. It is interesting that today the same country that produced 110 000 tons of tannin extract in 1946, now only produces 42 000, only half of which are still used for leather manufacture.

As a consequence of the steady dwindling in tannin sales for leather, in the 1960s and 1970s the industry started to desperately look for new applications for these natural products. After all, they had survived the collapse of the silk dyes market more than a century earlier, and a new lease of life could perhaps be found in other fields. Many applications were tried, from varnish primers for metals, which effectively were in use in Britain for some time during the 1960s and 1970s, to antipollution flocculating agents that were successful for about 15 years in the 1970s and 1980s, before being superseded by better synthetic materials [18]. About 600 tons tannin per year, are still in use as ore flotation agents, especially in a couple of feldspar mines in southern Africa [18]. Furthermore, fluidifying agents for drilling mud and superplasticizing additives for cement were developed [101]. To the best knowledge of the author, about 70–80 tons per year of tannin-based cement additives are still used. The main use found, however, was for tannin adhesives for wood panels and other wood products. Production started in 1973 and reached 4 500 dry tons/year of tannin by 1978, and this figure has now risen to around 25 000.

The application of tannins as wood adhesives contributed to save a few tannin extraction factories and to stabilize the situation in some major producing countries, but did not translate into an immediate and definitive rescue of that industry. Worldwide, leather still consumes more tannin than adhesive formulations. This is because oil-derived synthetic adhesives are cheaper than the tannin-based counterparts. The first oil crisis of 1974 at least convinced a few southern hemisphere industrialists (in Australia, South Africa and New Zealand) to use tannin adhesives to avoid the difficulties of supplying synthetic adhesives. The phenomenon persisted with a couple of still existing exceptions, but it did not take off in Europe or North America. Synthetic adhesives became cheap again and in abundant supply after 1974, being strongly 'pushed' by all the big chemical companies. Considerable renewed interest in tannins started again after the year 2000 as a result of two factors: (i) the recent marked increase in oil prices that raised disproportionately the market price of all synthetic adhesives, thus favouring natural raw materials. As a consequence, tannin has now become much cheaper than phenol and competes with the cheapest adhesive of them all, urea–formaldehyde resins; (ii) the recent severe tightening of formaldehyde emission regulations, mainly the introduction of the extremely severe Japanese standard, a regulation that is now starting to spill over into other countries [2, 3].

Although tannin adhesives are now fast becoming an interesting industrial proposition as an alternative to synthetic homologues, the use of tannins is expanding rapidly to an even more interesting field, namely to the pharmaceutical/medicine areas. Thus, a fourth market transformation has started and it appears set to overtake tannin transformation into wood adhesives, at least in terms of monetary added value. The therapeutic virtues of the addition of tannins in wine (consequence of the so-called French Paradox), the use of tannins to cure some gastrointestinal diseases, the increasing use of tannins as food supplements in North America, and the research on the beneficial effects of tannins in a multitude of diseases, even serious ones such as cancer and virus-induced sicknesses, are in full swing. This is because the value added to the base cost of tannins is considerable. Indeed, since tannins to be used for human consumption must obviously be thoroughly purified, their price is 40–50 times higher than that of tannins for industrial applications.

8.2 MAJOR SOURCES

The sources of tannins are very varied. There is a multitude of trees and shrubs which contain tannins. For both hydrolysable and condensed structures, the species rich in tannins are many. Notable for either their

present or past economic and/or industrial importance are black wattle or black mimosa bark (*Acacia mearnsii*), quebracho wood (*Schinopsis balansae or lorentzii*), oak bark (*Quercus* spp.), chestnut wood (*Castanea sativa*), mangrove wood, *Acacia catechù*, *Uncaria gambir*, sumach, myrabolans (*Terminalia* and *Phyllantus* tree species), divi-divi (*Caesalpina coraria*), algarobilla chilena, tara, and the bark of several species of pines and firs, among them *Pinus radiata* and *Pinus nigra*, not counting even more plants with extractable tannins.

As regards the tannin origin by location, the areas of strong industrial production today are Brazil, South Africa, India, Zimbabwe, Tanzania for mimosa tannin; Argentina for quebracho tannin; Indonesia for mangrove and for cube Gambier tannins; and Italy and Slovenia for chestnut tannin. There are many other small to very small producers a bit everywhere, for example, small pine tannin factories in Turkey and Chile, an oak tannin factory in Poland and a grape pip tannin factory in France.

8.3 USES

The variety of uses of tannins has been illustrated in the introduction. Some, which were important in the past but are no longer now, will not be described, as the literature on the subject is extant [1, 14, 15, 18]. The major existing uses or past uses of tannins are listed below, and the more important will be developed later in more detail.

(1) Leather manufacture.
(2) Adhesives, in particular wood adhesives.
(3) Wine, beer and fruit juices additives.
(4) Ore flotation agents.
(5) Cement superplasticizers.
(6) Medical and pharmaceutical applications.

Of these, leather manufacture will only be briefly discussed as it is beyond the scope of this review and because extensive technical and scientific literature is available on the subject [1, 5, 7, 14, 15–19, 27]. Suffice it to say that it is the interaction between the phenolic hydroxy groups of the tannins and the polar groups of proteins that give rise to a very strongly associated whole.

8.4 TANNIN STRUCTURE

The term natural vegetable tannins is used loosely to define two broad classes of chemical compounds of mainly phenolic nature, namely condensed or polyflavonoid tannins and hydrolysable tannins. The recognized oligomeric nature of condensed tannins [4–7] contrasts with the allegedly nonpolymeric nature of hydrolysable tannins [5–7].

8.4.1 Hydrolysable tannins

Hydrolysable tannins, including chestnut (*Castanea sativa*), myrabolans (*Terminalia* and *Phyllantus* tree species), divi-divi (*Caesalpina coraria*), tara, algarobilla, valonea, oak and several other commercial tannin extracts are reputed to be mixtures of simple phenols such as gallic and ellagic acids and of esters of a sugar, mainly glucose, with gallic and digallic acids, and with more complex structures containing ellagic acid (Fig. 8.1).

Notwithstanding their alleged lack of a polymeric nature, they can form complex structures. It must be noted first that carbohydrates are intimately and covalently linked to the phenolic moieties in the structure of

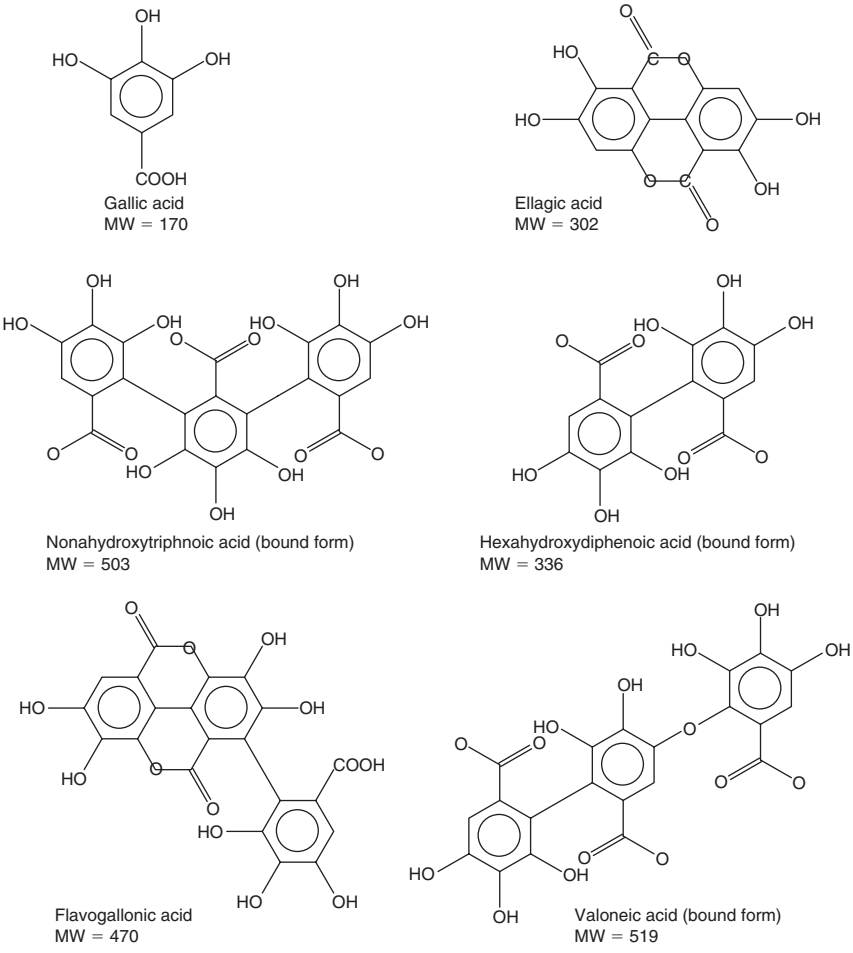
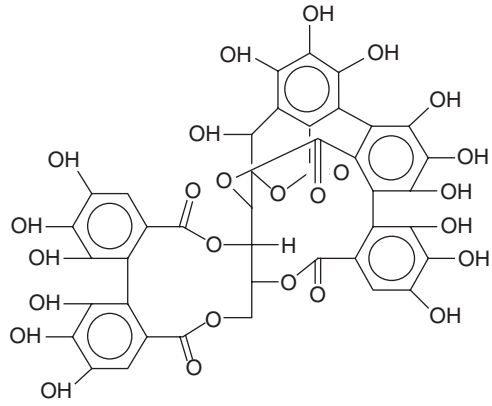


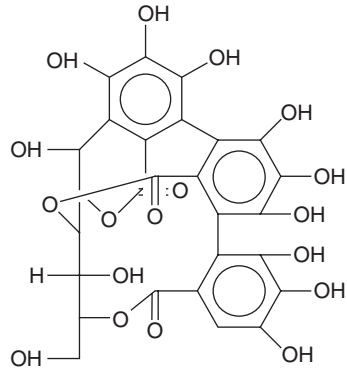
Figure 8.1 Chemical species characteristic of the low molecular weight fraction of hydrolysable tannins.

these tannins, and are therefore to be considered as part of the tannin itself. Indeed, several studies [4, 5] identified the major constituents of the main commercial hydrolysable tannin, chestnut tannin extract (an ellagitannin), as the positional isomers castalagin and vescalagin (**I**), present respectively in 14.2 and 16.2 per cent by mass.



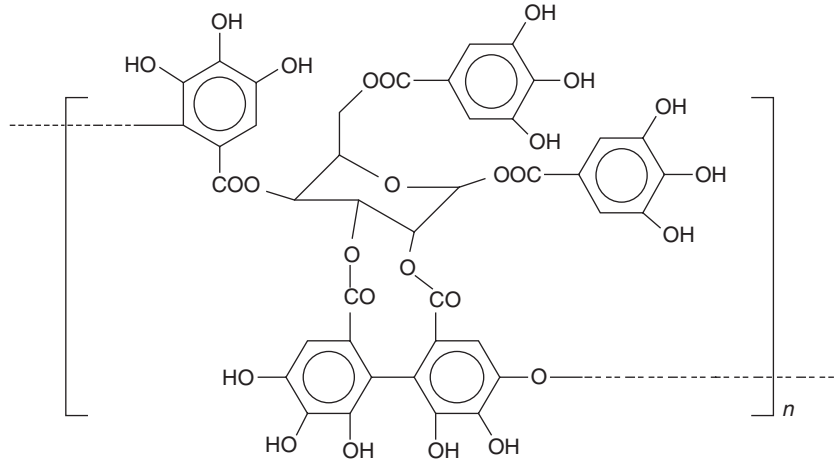
(I)

The rest of the tannin was found to contain 6.6 per cent of the positional isomers castalin and vescalin (**II**) [4, 5], 6 per cent of gallic acid, and 3 per cent of pentagalloyl glucose monomer.



(II)

It must be pointed out however that the authors of the study advancing relative composition percentages [5] clearly state that the disadvantage of the chromatographic technique they used is its strong adsorption of several types of tannins, particularly tannins composed of large molecules. This limitation might well slant the percentages of lower to higher molecular weight components in the analysis of the chestnut extract [4]. Notwithstanding such a limitation, two classes of compounds have however mass predominance in chestnut tannins, namely 28.8 per cent of small molecules, the formula of which is shown in Fig. 1, and 25.4 per cent (or higher, see reasons above) of an unknown and difficult to isolate fraction of apparently a very much higher molecular weight [5] and a very low TLC Rf. This fraction appears to be composed of a number of closely related components giving a continuous TLC smudge of Rf values between 0 and 0.33, which has recently been identified as a mixture of oligomers of pentagalloyl glucose [8], leading to the hypothesis that castalagin, vescalagin, vescalin and castelin are nothing but the hydrolysis of the real structure of the tannin as present in nature [8]. Circumstantial evidence strongly suggests that their repeat unit is:

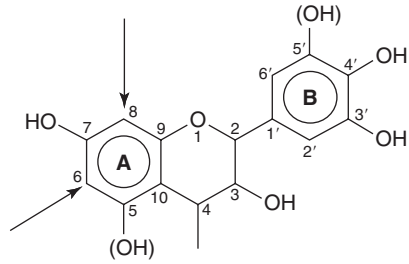


Although these tannins can be reacted with formaldehyde and other aldehydes, the rates of these interactions are low, and they are therefore not favoured for the preparation of resins. They have, however, been used successfully as partial substitutes (up to 50 per cent) of phenol in the manufacture of phenol–formaldehyde resins [9, 10]. Their chemical behaviour towards formaldehyde is analogous to that of simple phenols of low reactivity and their moderate use as phenol substitutes in the above-mentioned resins does not present difficulties. Their lack of macromolecular structure, the low level of phenol substitution they allow, their low nucleophilicity, limited worldwide production and relatively high price, somewhat decrease their chemical and economical interest for resin production. Consequently, their main use is for leather tanning where their performance, especially in terms of clarity of colour and light resistance, is truly excellent.

8.4.2 Condensed (polyflavonoid) tannins

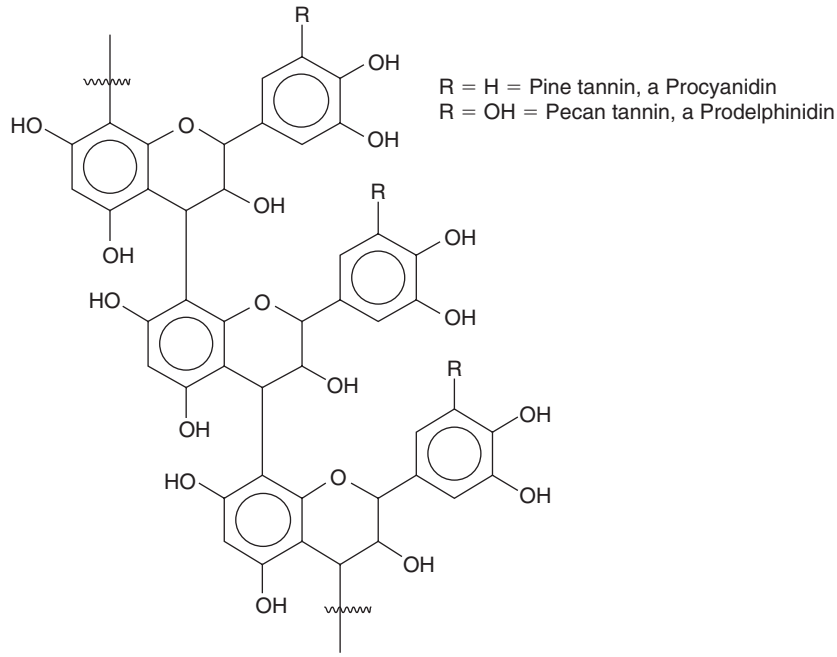
Condensed tannins constitute more than 90 per cent of the total world production of commercial tannins (200 000 tons per year) [11]. Their high reactivity towards aldehydes and other reagents renders them both chemically and economically more interesting for the preparation of adhesives, resins and other applications apart from leather tanning. The main commercial species, such as mimosa and quebracho, also yield excellent heavy duty leather. Condensed tannins and their flavonoid precursors are known for their wide distribution in nature and particularly for their substantial concentration in the wood and bark of various trees. These include various *Acacia* (wattle or mimosa bark extract), *Schinopsis* (quebracho wood extract), *Tsuga* (hemlock bark extract), *Rhus* (sumach extract) species, and various *Pinus* bark extract species, from which commercial tannin extracts are manufactured.

The structure of the flavonoid constituting the main monomer of condensed tannins may be represented as follows:



This flavonoid unit is repeated 2–11 times in mimosa and quebracho tannins [11, 12], with an average degree of polymerization of 4–5, and up to 30 times for pine tannins, with an average degree of polymerization of 6–7 for their soluble extract fraction [13]. The nucleophilic centres on the A-ring of a flavonoid unit tend to be more reactive in aromatic substitution, than those found on the B-ring. This is due to the vicinal hydroxyl substituents, which cause general activation in the B-ring, without any localized effects such as those found in the A-ring [11].

The following three-unit fragment illustrates two typical tannin structures:



Formaldehyde and other aldehydes react with tannins to induce polymerization through methylene bridge linkages at reactive positions on the flavonoid molecules, mainly the A-rings. The reactive positions of the A-rings are

the 6 *or* 8 locations (according to the type of tannin) of all the flavonoid units, and both of them for the upper terminal flavonoid units. The A-rings of mimosa and quebracho tannins show reactivity towards formaldehyde comparable to that of resorcinol [11, 14–17]. Assuming the reactivity of phenol to be 1 and that of resorcinol to be 10, the A-rings have a reactivity of 8–9. However, because of their size and shape, the tannin molecules lose their mobility and flexibility at a relatively low level of condensation with formaldehyde, so that the available reactive sites are too far apart for further methylene bridge formation. The result may be incomplete polymerization and therefore poor material properties. Bridging agents with longer molecules should be capable of joining the distances that are too long for methylene bridges. Alternatively, other techniques can be used to solve this problem [11, 100].

In condensed tannins from mimosa bark, the main polyphenolic pattern is represented by flavonoid analogues based on resorcinol A-rings and pyrogallol B-rings. These constitute about 70 per cent of the tannins. The secondary but parallel pattern is based on resorcinol A-rings and catechol B-rings [11, 14]. These tannins represent about 25 per cent of the total of mimosa bark tannin fraction. The remaining parts of the condensed tannin extract are the 'nontannins' [14]. They may be subdivided into carbohydrates, hydrocolloid gums and small amino and imino acid fractions [11, 14]. The hydrocolloid gums vary in concentration from 3 to 6 per cent and contribute significantly to the viscosity of the extract despite their low concentration [11, 14]. Similar flavonoid A- and B-ring patterns also exist in quebracho wood extract (*Schinopsis balansae*, and *Lorentzii*) [15–17], but no phloroglucinol A-ring pattern, or probably a much lower quantity of it, exists in the quebracho extract [17–19]. Similar patterns to wattle (mimosa) and quebracho are encountered in hemlock and Douglas fir bark extracts. Completely different patterns and relationships are found instead in pine tannins [20–22] which present only two main patterns: one represented by flavonoid analogues based on phloroglucinol A-rings and catechol B-rings [20, 22] and the other, present in a much lower proportion, represented by phloroglucinol A-rings and phenol B-rings [20, 22]. The A-rings of pine tannins then possess only the phloroglucinol type of structure, much more reactive towards formaldehyde than a resorcinol-type counterpart, with important consequences in the use of these tannins for adhesives.

8.5 ANALYSIS

Various methods of analysis are available for the determination of tannin content. These methods can generally be grouped into two broad classes:

(1) *Methods aimed at the determination of tannin material content in the extract*: The classical method of this type still used is the hide-powder method. These methods were devised to determine which percentage of the extract would participate in leather tanning. Their main drawback for their use in adhesives is their incapacity of detecting and determining the approximate 3–6 per cent of monoflavonoids and biflavonoids, or phenolic 'nontannins', present in the extract, which do not contribute to tanning capacity, but which do definitely react with formaldehyde and contribute to adhesive preparation.

(2) *Methods aimed at the determination of phenolic materials present in the extract that can be reacted with formaldehyde*: These methods were devised particularly for tanning extract used in adhesives and are all based on the determination of some of the products of the reaction of the flavonoids with formaldehyde.

The accepted methods of the first type comprise the hide-powder method [23], the refractometric method and various visible ultraviolet, and infrared spectroscopic methods. The accepted methods of the second type include comparative methods such as the Stiasny–Orth method [24, 25] and its modifications, all these being gravimetric methods today largely obsolete due to the lack of reliability consequent to the coprecipitation of some carbohydrates together with the phenolic material of the tannin extract and to the results being expressed in an absolute value, which is never convertible to a percentage of useful material in the extract. The Lemme sodium bisulphite backtitration method [26], the ultraviolet spectrophotometric molibdate ion method [27], and the infrared spectrophotometric methods [28] give instead correct percentage results.

8.6 A FEW CONSIDERATIONS ON LEATHER MANUFACTURE

The leather tanning industry is one of the most ancient processes still in operation. Extensive reviews and articles on the use of vegetable tannins in leather making exist [1, 5, 7, 14, 15–19, 27]. A brief general and comparative outline

of vegetable versus other types of tanning is given here for the information of the reader who might not know this industry. Although the technology of leather manufacture has evolved over centuries, and even in recent years, the basic principles for the production of leather have remained essentially the same. Hide proteins, mainly collagen, are rendered insoluble and dimensionally more stable by treatment with chemical products able to join them and thus render them both more resistant to mechanical wear and less susceptible to biological degradation and other types of attack. The main products used today for leather tanning are (i) acid salts of trivalent chromium, mainly used for the manufacture of soft leathers for shoe uppers and for leather bags; (ii) forestry-derived, natural vegetable tannins, such as chestnut and flavonoid extracts, mainly used for the manufacture of heavy, rigid and hard leathers for shoe soles, saddles, belts and other implements subject to intensive wear; (iii) aldehydes, in particular formaldehyde and glutaraldehyde; (iv) sulphonated synthetic polymers, such as acid phenol–formaldehyde novolak-type resins and (v) a number of other synthetic resins and compounds (acrylics, oxazolidines, aminoplastic resin, etc.).

Each of the products mentioned above is more apt than the others to the manufacture of certain specific types of leather. The fact remains however, that the first two in the above list account for more than 90 per cent of all the leather manufactured today, and that the process based on trivalent chromium salts accounts by itself for about 70 per cent of the total. Chrome tanning is particularly suited for soft leather, as it does not affect hide flexibility and renders the leather very lightfast and very stable both chemically and physically. It produces leather of excellent antishrinkage ability, as indicated by its high shrinkage temperature in testing. The forestry-derived vegetable tannins have, instead, a strong astringent effect (they fix very effectively on the collagen structure) and give considerable 'body', hardness and toughness to the leather produced with them. However, they have the considerable disadvantage of exhibiting marked darkening problems when exposed to light and, even worse, to shrink at a much lower temperature. It is these two main disadvantages that have somewhat limited their application in relation to the ubiquitousness of chromium salts. Conversely, their combination with some synthetic resins such as MUF [29, 30], give light-coloured leathers presenting high resistance to light-induced degradation, as well as other advantages similar to those of chrome tanning.

Chromium salts are becoming less acceptable in many types of industry due to potential effluent pollution. Furthermore, well-defined quality standards as regards leather product skin-contact allergic reactions, have also been introduced for finished products, for instance in leather clothing and interior car linings. In this respect, two of the requirement limits to comply with are the amount of both leachable trivalent chromium, which generally does not constitute a problem, and of one of its tanning derivatives, namely the more dangerous, highly toxic hexavalent chromium salts. Recent norms [31, 32] limit severely the proportion of these compounds in leathers to be used in direct contact with human skin, such as watch straps, shoe uppers, etc.). Furthermore, the treatment of tanning waste waters represents one of the major problems in the leather industry, especially today that the relevant European Commission norms impose ever more stringent effluent limits. The waste waters are generally treated to abate (never eliminate) chromium salt residues. However it has proved difficult to find suitable alternatives to chromium salts up to now.

In the case of natural tannins, their sensitivity to photo-oxydation limits their use to applications where such a characteristic is of no consequence. It is the tannin phenolic structure itself which renders photo-oxydation possible [33], with transformation of tannin phenolic groups to coloured quinones, but it has been shown that this effect can be drastically limited if the tannins are condensed with sulphonated synthetic aminoplast resins such as MUF [29, 30]. Conversely, while the use of synthetic aminoplast resins is developing in the tanning industry, as they give leathers of a certain degree of softness and flexibility, and particularly suitable for colouring, their weak point is the excessive and inevitable presence of free formaldehyde [34] and their poor tanning capability due to their low astringency. Polyphenolic vegetable tannins are well known to act as powerful free formaldehyde scavengers, as they react rapidly and irreversibly with this compound [11, 35]. Their combination with aminoplast MUF resins thus reduces markedly the photo-oxydation of vegetable tannins through synergy with the synthetic resin, reduces formaldehyde emission to just about zero, yields relatively soft but also tough leather and eliminates the need for chromium salts. Furthermore, such a mix can achieve leather shrinkage temperatures that at least match the chromium salt performance on this parameter.

8.7 TANNIN-BASED ADHESIVES

8.7.1 Wood adhesives

In condensed polyflavonoid tannin molecules, the A-rings of the constituent flavonoid units retain only one highly reactive nucleophilic centre, the remainder accommodating the interflavonoid bonds. Resorcinolic A-rings (wattle) show

reactivity towards formaldehyde comparable to, though slightly lower than, that of resorcinol [36]. Phloroglucinolic A-rings (pine) behave instead as phloroglucinol [37]. Pyrogallol or catechol B-rings are by comparison unreactive and may be activated by anion formation only at relatively high pH [38]. Hence the B-rings do not participate in the reaction except at high pH values (pH 10), where the reactivity towards formaldehyde of the A-rings is so high that the ensuing tannin–formaldehyde adhesives have unacceptably short pot lives [36]. In the usual tannin adhesive practice, only the A-rings are used to generate the network. With regard to the pH dependence of the reaction with formaldehyde, it is generally accepted that the reaction rate of wattle tannins with formaldehyde is the slowest in the pH range 4.0–4.5 [39]; for pine tannins, the range is between 3.3 and 3.9.

Formaldehyde is generally the aldehyde used in the preparation, setting and curing of tannin-based adhesives. It is normally added to the tannin extract solution at the required pH, preferably in its polymeric form of paraformaldehyde, which is capable of fairly rapid depolymerization under alkaline conditions, and as urea–formalin concentrates. Hexamethylenetetramine (hexamine) may also be added to these resins because of its potential formaldehyde releasing action under heat. Hexamine is, however, unstable in acid media [40], but becomes more stable with increasing pH values. Hence, under alkaline conditions, the liberation of formaldehyde might not be as rapid and as efficient as wanted. Also, it has been fairly widely reported, with a few notable exceptions [41], that bonds formed with hexamine as hardener are not as boil resistant [42] as those formed by paraformaldehyde. The reaction of formaldehyde with tannins may be controlled by the addition of alcohols to the system. Under these circumstances, some of the formaldehyde is stabilized by the formation of hemiacetals (*e.g.* $CH_2(OH)(OCH_3)$) if methanol is used [11, 37]. When the adhesive is cured at an elevated temperature, the alcohol is driven off at a fairly constant rate and formaldehyde is progressively released from the hermiacetal. This ensures that less formaldehyde is volatilized when the reactants reach curing the curing temperature and that the pot life of the adhesive is extended. Other aldehydes have also been substituted for formaldehyde [11, 36, 39, 41].

In the reaction of polyflavonoid tannins with formaldehyde two competitive mechanisms take place:

(1) The reaction of the aldehyde with tannin and with low molecular weight tannin–a1dehyde condensates, which is responsible for the aldehyde consumption.
(2) The liberation of formaldehyde, which become available again for reaction. This release is probably due to the decomposition of the unstable —CH_2—O—CH_2— ether bridges initially formed into —CH_2— counterparts.
(3) In the case of some tannins (*e.g.* quebracho tannin) a third important reaction occurs, namely the simultaneous hydrolysis of some interflavonoid bonds, hence a depolymerization reaction, partly counteracting and thus slowing down the hardening process [12, 42, 43].

Considering the fact that the two major existing industrial polyflavonoid tannins, namely mimosa and quebracho tannins, are very similar and both composed of mixed prorobinetinidins and profisetinidins, it was difficult to rationalize this anomalous behaviour of quebracho tannin. It has now been possible to determine by both NMR [42] and particularly by laser desorption mass spectrometry (MALDI–TOF), applied to mimosa and quebracho tannins and some of their modified derivatives [12] that: (i) mimosa tannin is predominantly composed of prorobinetinidins, while quebracho is predominantly composed of profisetinidins; (ii) mimosa tannin is heavily branched due to the presence of considerable proportions of 'angular' units in its structure, while quebracho tannin is almost completely linear [12]. This latter structural difference is the one which contributes to the considerable differences in viscosity of water solutions of the two tannins and which induces the interflavonoid link of quebracho to be more easily hydrolysable, because of the linear structure of this tannin. This feature confirms the NMR findings [12, 42] showing that this tannin is subject to polymerization/depolymerization equilibria. The specificity of the quebracho structure also explains the decrease in viscosity arising from acid/base treatments to yield tannin adhesive intermediates after a certain level of hydrolysis of the tannin itself and not only of the carbohydrates present in the extract (see also Section 8.4). Such a tannin hydrolysis does not appear to occur with mimosa tannin in which the interflavonoid link is completely stable against this attach.

It is interesting to note that whereas —CH_2—O—CH_2— ether bridged compounds have been isolated for the phenol–formaldehyde [40] reaction, their existence for fast-reacting phenols such as resorcinol and phloroglucinol has been postulated, but they have never been isolated, since these two phenols have always been considered too reactive towards formaldehyde. They are detected indirectly by the surge in the concentration of formaldehyde observed in kinetic studies, as a consequence of the methylene ether bridge decomposition [44].

When heated in the presence of strong mineral acids, condensed tannins are subject to two competitive reactions. One is degradative leading to lower molecular weight products, and the second is condensative, as a result of

the hydrolysis of heterocyclic rings (*p*-hydroxybenzyl ether links) [38]. The generated *p*-hydroxybenzylcarbenium ions condense randomly with nucleophilic centres on other tannin units to form 'phlobaphenes' or 'tanner's red' [38, 45–47]. Other modes of condensation (*e.g.* free radical coupling of B-ring catechol units) cannot be excluded in the presence of atmospheric oxygen. In predominantly aqueous conditions, the formation of phlobaphene or insoluble condensates predominates. These reactions, characteristic of tannins and not of synthetic phenolic resins, must be taken into account when formulating tannin adhesives.

The sulphitation of tannins is one of the oldest and most useful reactions in flavonoid chemistry. Slightly sulphited water is sometimes used to increase tannin extraction from the bark containing it. In certain types of adhesives, the total effect of sulphitation has both negative and positive connotations. The latter aspects are related to both a higher concentration of tannin phenolics in adhesive applications, due to enhanced solubility and decreased viscosity, and to a higher moisture retention by the tannin resins, giving a slower adhesive film dry-out and hence a longer assembly time [48]. As for the negative aspects, the presence of sulphonate groups promotes a higher sensitivity to moisture with a consequent adhesive deterioration and bad water resistance of the cured glue line even with adequate crosslinking [48–51].

In recent years, the importance of the marked colloidal nature of tannin extract solutions has come to the fore [42, 43, 52–60]. It is the presence of both polymeric carbohydrates in the extract, as well as of the higher molecular fraction of the polyphenolic tannins, which determines the colloidal state of tannin extract solutions in water [42, 52]. This feature affects many of the reactions which lead to the formation and curing of tannin adhesives, to the point where reactions not thought possible in solution become instead, not only possible, but, the favoured ones [42, 52]. Conversely, reactions mooted to be of determinant importance when found on models not in a colloidal state, have in reality been shown to be inconsequential to tannin adhesives and their applications [35, 59].

8.8 TECHNOLOGY OF INDUSTRIAL TANNIN ADHESIVES

8.8.1 Wood adhesives

The purity of vegetable tannin extracts varies considerably. Commercial wattle bark extracts normally contain 70–80 per cent active phenolic ingredients. The nontannin fraction, consisting mainly of simple sugars and high molecular weight hydrocolloid gums, does not participate in the resin formation with formaldehyde. Sugars reduce the strength and water resistance in direct proportion to the amount added. Their effect is a mere dilution effect of the adhesive resin solids, with the consequent proportional worsening of adhesive properties. The hydrocolloid gums, instead, have a much more marked effect on both original strength and water resistance of the adhesive [11, 37, 58]. If it is assumed that the nontannins in tannin extracts have a similar influence on adhesive properties, it can be expected that unfortified tannin–formaldehyde networks can achieve only 70–80 per cent of the performance shown by synthetic adhesives.

In many glued wood products, the demands on the glue line are so high that unmodified tannin adhesives are unsuitable. The possibility of refining extracts has proved fruitless largely because the intimate association between the various constituents makes industrial fractionation difficult. Fortification is in many cases the most practical approach to reducing the effect of impurities and generally consists of copolymerization of the tannin with phenolic or aminoplastic resins [36, 37, 58, 61]. It can be carried out during the manufacture of the adhesive resin, during gluemix assembly, just before use, or during adhesive use. If added in sufficient quantity, various synthetic resins have been found effective in reducing the nontannin fraction to below 20 per cent and in overcoming other structural problems [36, 37]. The main resins used are phenol–formaldehyde and urea–formaldehyde resols with a medium-to-high methylol group content. These resins can fulfil the functions of hardeners, fortifiers, or both. Generally, they are used as fortifiers in between 10 and 20 per cent of total adhesive solids, with paraformaldehyde used as a hardener. Such an approach is the favourite one for marine-grade plywood adhesives. These fortifiers are particularly suitable for the resorcinol types of condensed tannins, such as mimosa. They can be copolymerized with the tannins during resin manufacture, during use, or both [11, 35–37, 58]. Copolymerization and curing are based on the condensation of the tannin with the methylol groups carried by the synthetic resin. Since tannin molecules are generally large, the rate of molecular growth in relation to the rate of linkage is high, so that tannin adhesives generally tend to have fast gelling and curing times and shorter pot lives than those of

synthetic phenolic adhesives. From the point of view of reactivity, phloroglucinol tannins, such as pine tannins, are much faster than mainly resorcinol tannins such as mimosa. The usual ways of slowing them down and, for instance, lengthening the adhesive pot life are:

(1) To add alcohols to the adhesive mix to form hemiacetals with formaldehyde which therefore act as retardants of the tannin–formaldehyde reaction.
(2) To adjust the adhesive pH to obtain the required pot life and rate of curing.
(3) To use hexamine as a hardener which, under the current conditions, gives a very long pot life at ambient temperature but still a fast curing time at higher temperatures.

The viscosity of bark extracts is strongly dependent on concentration and increased very rapidly above 50 per cent. Compared to synthetic resins, tannin extracts are more viscous at the concentrations normally required in adhesives. The high viscosity of aqueous solutions of condensed tannins is due to the following causes, in order of importance:

(1) *Presence of high molecular weight hydrocolloid gums in the tannin extract* [58, 60]: The viscosity is directly proportional to the amount of gums present in the extract [58, 60].
(2) *Tannin–tannin, tannin–gum and gum–gum hydrogen bonds*: Aqueous tannin extract solutions are not true solutions but, rather, colloidal suspensions in which water access to all parts of the molecules present is very slow. As a consequence, it is difficult to eliminate intermolecular hydrogen bonds by dilution only [58, 60].
(3) *Presence of high molecular weight tannins in the extract* [12, 58, 60].

The high viscosity of tannin extract solutions has also been correlated to the proportion of very high molecular weight tannins present in the extract. This effect is not well defined. In most adhesive applications, such as in plywood adhesives, the viscosity is not critical and can be manipulated by dilution.

In the case of particleboard adhesives, the decrease in viscosity is, instead, an important prerequisite. When reacted with formaldehyde, unmodified condensed tannins give adhesives having characteristics that do not suit particleboard manufacture, namely, high viscosity, low strength and poor water resistance. The most commonly used process to eliminate these disadvantages in the preparation of tannin-based particleboard adhesives consists of a series of subsequent acid and alkaline treatments of the tannin extract, causing hydrolysis of the gums to simple sugars and some tannin structural changes, thus improving the viscosity, strength and water resistance of the unfortified tannin–formaldehyde adhesive [11, 35]. Furthermore, such treatments may cause the partial rearrangement of the flavonoid molecules that causes liberation of some resorcinol *in situ* in the tannin, rendering it more reactive, allowing better crosslinking with formaldehyde and, ultimately, yielding an adhesive which, without addition of any fortifier resins, gives a truly excellent performance for exterior-grade particleboard [11, 35]. This modification cannot be carried out too extensively, in order to avoid the precipitation of the tannin from the solution by the formation of 'phlobaphenes'.

Typical results are shown in Table 8.1.

Table 8.1

Unfortified tannin–formaldehyde adhesives obtained by acid–alkali treatment, for exterior-grade particleboard: Example of industrial board results [11, 77, 78]

	Swelling after a 2 h boil				
Panel density (g/cm^3)	Measured wet (%)	Measured dry (irreversible swelling) (%)	Original internal bond (IB) tensile perpendicular (kg/cm^2)	IB after a 2 h boil (kg/cm^2)	Cyclic test after five cycles measured (%)
0.700	11.0	0.0	13.0	9.0	3.0

Particular gluing and pressing techniques have been developed for tannin particleboard adhesives [62, 63] to achieve pressing times much shorter than those traditionally obtained with synthetic phenol–formaldehyde adhesives, although recent advances in the latter materials have markedly limited such an advantage [64, 65]. Pressing times of 7 s mm^{-1} of panel thickness have been achieved and of 9 s mm^{-1} at 190–200°C pressing temperatures are in daily operation: these are pressing times that are becoming comparable to those obtainable with urea–formaldehyde or melamine–formaldehyde resins at the same pressing temperatures. The success of these simple types of particleboard adhesives relies heavily on industrial application technologies rather than just on the preparation technology of the adhesive itself [58, 62, 66]. A considerable advantage is the much higher moisture content of the resinated chips tolerable with these adhesives than with any of the synthetic phenolic and aminoresin counterparts. In the case of wood particleboard and of oriented strandboard (OSB) panels, the technology so developed allows hot-pressing at moisture contents of around 24 per cent, against values of 12 per cent for traditional synthetic adhesives, and presents other advantages as well [58, 66, 67].

The best adhesive formulation for phloroglucinolic tannins, such as pine tannin extracts is, instead, a comparatively new and is also capable of giving excellent results when using resorcinol tannins such as a wattle tannin extract [68–71]. The adhesive gluemix consists only of a mix of an unmodified tannin extract 50 per cent solution to which paraformaldehyde and polymeric nonemulsifiable 4,4′-diphenylmethane diisocyanate (commercial pMDI) are added [68–71]. The proportion of tannin extract solids to pMDI can be as high as 70/30 w/w, but can be much lower in pMDI content. This adhesive is based on the peculiar mechanism by which the pMDI in water, is hardly deactivated to polyureas because it reacts faster with the hydroxymethyl groups of a formaldehyde-based resin, be it a tannin or another resin [69, 71].

The properties of the particleboards manufactured with this system using pine tannin adhesives are listed in Table 8.2. The results obtained with this system are quite good and not too different from those produced by some of the other tannin adhesives already described. In the case of phloroglucinolic tannin extracts, no pH adjustment of the solution is needed. One point that was given close consideration is the deactivating effect of water on the isocyanate group of pMDI. It has been found that the amount of deactivation by water in a concentrated solution (50 per cent or over) of a phenol is much lower than previously thought [68–71]. This is the reason why aqueous tannin extract solutions and pMDI can be mixed without any substantial pMDI deactivation by the water present.

The quest to decrease or completely eliminate formaldehyde emission from wood panels bonded with adhesives, although not really necessary in tannin adhesives due to their very low emission (as with most phenolic adhesives), has nonetheless promoted here too some research to further reduce formaldehyde emission. This has centred into two lines of investigation: (i) tannin autocondensation (see paragraph D later on), and (ii) the use of a hardener not emitting at all, simply because no aldehyde has been added to the tannin [72–74]. Methylolated nitroparaffins and, in particular, the simpler and least expensive exponent of their class, namely trishydroxymethyl nitromethane [72, 73], function well as hardeners for a variety of tannin-based adhesives, while affording considerable side advantages to the adhesive and to the bonded wood joint. In panel products such as particleboard, medium density fibreboard and plywood, the joint performance which is obtained is of the exterior/marine-grade type, combined with a very advantageous and very considerable lengthening in gluemix pot life is obtained. Furthermore, the use of this hardener is coupled with such a marked reduction in formaldehyde emission from the bonded wood panel that it is limited exclusively to the formaldehyde released only by the heated wood, or even less, thus functioning as a mild depressant of emission from the wood itself. Moreover, trishydroxymethyl nitromethane can be mixed in any proportion with traditional formaldehyde-based hardeners for tannin adhesives, its proportional substitution of such hardeners inducing a correspondingly marked decrease in the formaldehyde emission of the wood panel, without affecting its exterior/marine-grade

Table 8.2

Properties of a particleboard manufactured using pine tannin adhesives

Panel density	Swelling after a 2 h boil		Original internal bond (IB), tensile perpendicular (kg/cm^2)	IB after a 2 h boil (kg/cm^2)	IB retention after a 2 h boil (%)
	Measured wet (%)	Measured dry (irreversible swelling) (%)			
0.690	15.0	4.3	8.4	4.3	51

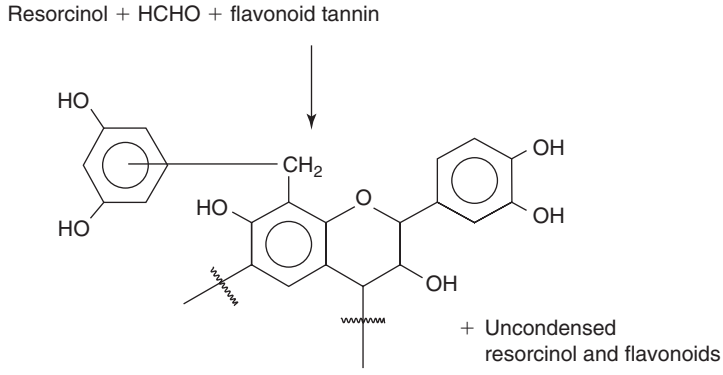
performance. Medium density fibreboard (MDF) industrial plant trials confirmed all the properties reported above [72, 73]. A cheaper but equally effective alternative to hydroxymethylated nitroparaffins is the use of hexamine as tannin hardener. This sometimes causes problems of an early agglomeration in some tannins [74] and a better solution which overcames this drawback was to use a mixture of formaldehyde and an ammonium salt as the hardener.

8.8.2 Corrugated cardboard adhesives

The adhesives developed for the manufacture of damp-ply-resistant corrugated cardboard are based on the addition of spray-dried wattle extract, urea–formaldehyde resin, and formaldehyde to a typical Stein–Hall starch formula with 18–22 per cent starch content [75, 76]. The wattle tannin–urea–formaldehyde copolymer formed *in situ* and any free formaldehyde left in the glue line are absorbed by the wattle tannin extract. The wattle extract powder should be added at level of 4–5 per cent of the total starch content of the mix (*i.e.* carrier plus slurry). Successful results can be achieved in the range of 2–12 per cent of the total starch content, but 4 per cent is the recommended starting level. The final level is determined by the degree of water hardness and desired bond quality. This wattle extract UF-fortifier system is highly flexible and can be adapted to damp-proof a multitude of basic starch formulations.

8.8.3 Cold-setting laminating and fingerjointing adhesives for wood

A series of different novolak-like materials are prepared by copolymerization of resorcinol with resorcinol A-rings of polyflavonoids, such as condensed tannins [77–79]. The copolymers formed have been used as cold-setting exterior-grade wood adhesives, complying with the relevant international specifications. Several formulations are used. The system most commonly employed commercially relies on the simultaneous copolymerization of resorcinol and of the tannin resorcinol A-ring, thanks to their comparable reactivity towards formaldehyde. The following scheme summarizes the principle of this system:



The final mixture is an adhesive that can be set and cured at ambient temperature by the addition of paraformaldehyde. Other cold-set systems exist and are described in the more specialized literature [11, 77–79]. Some typical results obtained with these adhesives are given in Table 8.3.

Table 8.3

Typical results of tannin–resorcinol–formaldehyde cold-setting adhesives used on beech strips according to British Standard BS 1204 [80]

	Dry	After 24 h cold-water soak	After 6 h boil
Tensile strength (N)	3 200–3 800	2 300–2 900	2 200–2 800
Wood failure (%)	90–100	75–100	80–100

A particularly interesting system now used extensively in several southern hemisphere countries is the so-called honeymoon' fast-setting, separate-application system [81, 82]. In this process, one of the surfaces to be mated in the joint is spread with a standard synthetic phenol–resorcinol–formaldehyde adhesive plus a paraformaldehyde hardner. The second surface is spread with a 50 per cent tannin solution at pH 12. When the two surfaces are jointed, fingerjoints develop enough strength to be installed within 30 min and laminated beams (glulam) need to be clamped for only 2.5–3 h instead of the traditional 16–24 h, with a consequent considerable increase in factory productivity. This adhesive system also provides full weather- and boil-proof capabilities.

8.8.4 TYRE CORD ADHESIVES

Another application of condensed tannin extracts that has proved technically successful is as a tyre cord adhesive. Both thermosetting tannin formulation [83] and tannin–resorcinol–formaldehyde formulations have been tested successfully.

8.9 NEW CONCEPTS AND PRINCIPLES

8.9.1 Surface catalysis

As in the case of other formaldehyde-based resins, the interaction energies of tannins with cellulose obtained by molecular mechanics calculations [59] tend to confirm the effect of surface catalysis induced by cellulose also on the curing and hardening reaction of tannin adhesives. The considerable energies of interactions obtained can effectively explain a weakening of the heterocycle ether bond leading to the accelerated and easier opening of the pyran ring in a flavonoid unit, as well as the ease with which hardening by self-condensation can occur. As in synthetic formaldehyde-based resins, the same effect explains the decrease in the activation energy of the condensation of polyflavonoids with formaldehyde leading to exterior wood adhesives curing and hardening [84].

8.9.2 Hardening by tannins self-condensation

The self-condensation reactions characteristic of polyflavonoid tannins have only recently been used to prepare adhesive polycondensates hardening in the absence of aldehydes [85]. This self-condensation reaction is based on the opening under alkaline and acid conditions of the O1—C2 bond of the flavonoid repeating unit and the subsequent condensation of the reactive centre formed at C2 with the free C6 or C8 sites of a flavonoid unit on another tannin chain [85–89]. Although this reaction may lead to considerable increases in viscosity, gelling does not generally occur. However, gelling does occur when the reaction takes place (i) in the presence of small amounts of dissolved silica (silicic acid or silicates) catalyst and some other catalysts [85–90], and (ii) on a lignocellulosic surface [89]. In the case of the more reactive procyanidin- and prodelphinidin-type tannins, such as pine tannin, cellulose catalysis is more than enough to cause hardening and to produce boards of strength satisfying the relevant standards for interior-grade panels [89]. In the case of the less reactive tannins, such as mimosa and quebracho, the presence of a dissolved silica or silicate catalyst is essential to achieve the panel strength required by the relevant standards. Self-condensation reactions have been shown to contribute considerably to the dry strength of wood panels bonded with tannins, but to be relatively inconsequential in contributing to the bonded-panel exterior-grade properties, which are instead attained by polycondensation reactions with aldehydes [89–91]. Combinations of tannin self-condensation and reactions with aldehydes, and combinations of radical and ionic reaction, have been used to decrease the proportion of aldehyde hardener needed, as well as to decrease considerably further the already low formaldehyde emission caused by the use of tannin adhesives [89–91].

8.10 CEMENT SUPERPLASTICIZERS

Plasticizers or dispersion agents are additives which are incorporated into concrete to improve its workability, reduce its water content needs, and enhance its strength development. Unfortunately, these properties are mutually

exclusive. Thus, all plasticizers also cause retardation in the setting and in the early strength development of concrete. Superplasticizers are, instead, substances that in small amounts are able to strongly fluidify a cement mix *without* retarding its setting. Heavily sulphonated melamine–formaldehyde resins are the only other superplasticizers known in cement technology, other than tannins. This enhanced workability without any addition of extra water entails neither a loss of final strength, nor any gross retardation of concrete strength. Furthermore, no decrease in initial strength is observed. Their remarkable plasticizing action is demonstrated by slumps of 200 mm without increases in water content or by water reduction of up to 30 per cent [92].

The effect of a superplasticizer is due to its sulphonic groups being oriented towards water, but also adsorbing on the cement grain surface in sufficient numbers to form a monolayer around the grain. The combination of electrostatic repulsion and large ionic size brings about a rapid dispersion of the individual cement grains. In doing so, water trapped within the original flocks is released and can contribute to the mobility of the cement paste and, hence, to the workability of the concrete. Superplasticizers do not cause much reduction in the surface tension of water. The adsorption of the anions on the surface of the cement grain is also less tenacious than in the case of retarders and the course of the hydration reaction is not hindered at normal dosage levels. It follows that, for normal superplasticizers, there is no significant retardation of setting and hardening.

Polyflavonoid tannins have structures capable of complexing metallic ions such as Fe^{2+}/Fe^{3+} and aluminium ions through the ortho hydroxyl groups of the B-ring of the flavonoid units [11]. They can also be sulphonated, and often are, to improve their solubility in water, with the consequent opening of their etherocyclic pyran ring and the introduction of sulphonic groups at the C2 sites of some of the flavonoid units [11]. They also contain up to 20 per cent monomeric and polymeric carbohydrates. Notwithstanding the well-known retarding effect of carbohydrates on cement, this is not the case in the presence of the tannins in the tannin extract. These characteristics render polyflavonoid tannins an interesting material for use as dispersing/plasticizing agents for cements, which are materials mostly composed of calcium and iron silicates and aluminates.

Sulphonated mimosa, quebracho and pine tannin extracts all behave well as cement superplasticizers, with mimosa and pine being the better ones [93]. A dosage of 0.25–0.5 per cent in cement has a noticeable effect of fluidification. The tannin extract cement superplasticizing behaviour was ascribed to the balance of different effects, namely (1) their increase in molecular weight by self-condensation induced by the presence of silicate and aluminium components of cement [72, 73, 93]; (2) the decrease first and then the stabilization of the molecular weight and the improved solubility induced by the introduction of sulphonic groups in the tannin structure and (3) the stabilization of the molecular weight induced by the addition of urea, through its hindrance to tannin self-condensation and its decrease of the tannin extracts colloidal association in water.

8.11 MEDICAL/PHARMACEUTICAL APPLICATIONS

Tannins are well known to have an antimicrobial activity. This is logical as their capability to tan proteins means that they will complex irreversibly also with the proteins in bacterial membranes, thus inhibiting any activity they might have. It follows that, pharmaceuticals containing tannins and aimed at curing bacterial intestinal infections have been around already for some time. Some studies on their anticavity effectiveness have also been conducted [94]. Additionally, the use of tannins in other pharmaceutical medical applications, have been reported, particularly concerning their antitumour and anticancer activity [95–98]. More recently, work on their antiviral effectiveness has been conducted [99]. The data in Tables 4 to 11 present preliminary results obtained on the antiviral activity of 12 different flavonoid and hydrolysable tannins, obtained at the medical department of Leuven University [99]. These results evaluated both the effectiveness of 12 different tannins, as measured by the Minimum Inhibitory Concentration (MIC) required to reduce virus-induced cytopathogenicity by 50 per cent. The lower the MIC value, the better the compound as an antiviral substance. Equally important, the results in the tables report the Minimum Cytotoxic Concentration (MCC) required to cause a microscopically detectable alteration in the normal cell morphology. The higher the MCC, the less toxic is the compound to the patient's cells and the better it is as an antiviral substance. Thus, what is sought is the lowest possible MIC and the highest possible MCC. These results were obtained as *in vitro* screening tests, which implies that *in vivo* conformations are required. Nonetheless, their novelty and thoroughness justify their full report here. Different tannins have been shown to be very effective against different viruses, the nature of the different polyphenolic groups being the cause of this behaviour. Most likely, they tan the proteins and associate with the carbohydrates of the virus membrane, through an interaction similar to that associating them with hide proteins and carbohydrates to give leather.

Table 8.4

Tannin concentration required to protect CEM cells against the cytopathogenicity of HIV by 50% (MIC)

Anti-HIV-1 and -HIV-2 activity of the compounds in human T-lymphocyte (CEM) cells		
Compound	EC_{50} (μg/ml)	EC_{50} (μg/ml)
	HIV-1	HIV-2
1. Mimosa tannin	6.0 ± 0.0	>20
2. Mimosa tanin intermediate [100]	5.0 ± 1.4	>20
3. Chestnut tannin	1.4 ± 0.5	>20
4. Tara + Chestnut mix	5.0 ± 1.4	>20
5. Quebracho standard	6.5 ± 0.7	>20
6. Quebracho highly purified	7.5 ± 0.7	>20
7. Quebracho highly sulphited	7.0 ± 1.4	>20
8. Pecan nut tannin	5.0 ± 1.4	>20
9. Cube Gambier	9.0 ± 1.4	>20
10. Radiata pine tannin	7.0 ± 1.4	>20
11. Maritime pine tannin	7.5 ± 0.7	>20
12. Sumach tannin	11.0 ± 1.4	>20
13. Spruce tannin	>100	>100

EC_{50} = effective concentration or concentration required to protect CEM cells against the cytopathogenicity of HIV by 50%

Table 8.5

Cytotoxicity and antiviral activity of compounds in HEL cell cultures, Herpes and vesicular stomatitis viruses. Tannins added prior to virus administration

Cytotoxicity and antiviral activity of compounds in E_6SM cell cultures						
Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μg/ml)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex $virus^{-1}$ TK^- KOS ACV^r
1	200	40	16	16	>80	40
2	⩾40	40	16	16	>80	40
3	⩾40	40	16	16	>80	47
4	⩾40	40	48	16	>80	47
5	40	47	36	16	>80	47
6	40	80	36	16	>80	47
7	⩾40	40	40	16	>80	36
8	40	16	16	16	>80	36
9	8	>80	>80	>80	>80	>80
10	40	40	>80	16	>80	40
11	40	>80	>80	80	>80	47
12	40	36	36	16	>80	36
13	40	>16	>16	>16	>16	>16
Brivudin	>400	0.128	400	16	>400	>400
Ribavirin	>400	>400	>400	400	>400	>400
Acyclovir	>400	0.384	0.128	>400	>400	48
Ganciclovir	>100	0.0064	0.0064	100	>100	2.4

[a] Required to cause a microscopically detectable alteration of normal cell morphology.

[b] Required to reduce virus-induced cytopathogenicity by 50%.

Table 8.6

Cytotoxicity and antiviral activity of compounds in HEL cell cultures, vesicular stomatitis, Coxsackie and respiratory syncytial viruses

Cytotoxicity and antiviral activity of compounds in HeLa cell cultures				
Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μ/ml)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
1	400	>80	>80	12 ± 5
2	400	>80	>80	12 ± 5
3	400	>80	>80	43 ± 4
4	400	43.4 ± 4	>80	35 ± 7
5	400	>80	>80	40 ± 0.2
6	400	>80	>80	40 ± 0.2
7	400	>80	>80	43 ± 5
8	400	>80	>80	9 ± 1
9	400	>80	>80	>80
10	400	>80	>80	40 ± 0.2
11	400	>80	>80	40 ± 0.2
12	80	>16	>16	>16
13	⩾80	>80	>80	>80
Brivudin	>400	>400	>400	>400
(S)-DHPA	>400	400	>400	>400
Ribavirin	>400	48	240	9.4

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

Table 8.7

Inhibitory effects of tannins on the proliferation of murine leukemia cells (L1210/0), murine mammary carcinoma cells (FM3A) and human T-lymphocyte cells (Molt4/C8, CEM/0)

Compound	IC_{50} (μg/ml)[a]			
	L1210/0	FM3A/0	Molt4/C8	CEM/0
1	18 ± 0	153 ± 66	74 ± 18	58 ± 0
2	16 ± 1	148 ± 74	66 ± 27	61 ± 1
3	17 ± 0	141 ± 7	98 ± 22	65 ± 2
4	17 ± 0	114 ± 1	75 ± 57	56 ± 0
5	12 ± 4	76 ± 16	20 ± 1	51 ± 30
6	15 ± 2	79 ± 27	33 ± 21	45 ± 27
7	14 ± 2	82 ± 26	40 ± 27	55 ± 25
8	21 ± 6	⩾200	81 ± 7	66 ± 11
9	13 ± 4	80 ± 22	17 ± 2	18 ± 1
10	65 ± 4	⩾200	65 ± 28	71 ± 9
11	53 ± 23	⩾200	94 ± 1	111 ± 40
12	17 ± 0	18 ± 1	17 ± 0	18 ± 2
13	49 ± 16	>200	145 ± 78	83 ± 20

[a] 50% inhibitory concentration.

Table 8.8

Cytotoxicity and antiviral activity of compounds in HEL cell cultures, influenza viruses. Compounds added prior to virus administration

Cytotoxicity and antiviral activity of compounds in MDCK cell cultures				
Compound	Minimum cytotoxic concentration[a] (μg/ml)	EC_{50}		
		Influenza A H1N1	Influenza A H3N2	Influenza B
		MTS	MTS	MTS
1	100	3.3 ± 1.2	1.7 ± 1.3	2.3 ± 1.9
2	100	2.2 ± 0.1	1.7 ± 0.6	2.3 ± 1.5
3	100	4.0 ± 2.8	2.0 ± 0	1.4 ± 0.8
4	33.3	4.1 ± 2.8	2.2 ± 0.8	1.4 ± 0.9
5	100	1.7 ± 0.1	2.1 ± 0.4	3.5 ± 3.2
6	100	5.4 ± 3.8	3.7 ± 1.6	3.6 ± 3.0
7	100	4.4 ± 2.8	1.9 ± 0.4	3.4 ± 3.0
8	33.3	2.1 ± 0.1	3.0 ± 1.5	1.8 ± 1.1
9	100	5.5 ± 4.6	4.4 ± 3.6	2.7 ± 2.6
10	100	4.2 ± 3.1	2.7 ± 1.0	2.7 ± 2.7
11	100	2.9 ± 1.2	2.2 ± 0.3	1.5 ± 1.1
12	20	2.0 ± 1.6	0.9 ± 0.2	2.6 ± 1.9
13	100	9.9 ± 5.7	9.5 ± 4.8	1.9 ± 1.9
Oseltamivir carboxylate (μM)	>100	0.05	0.65	10.65
Ribavirin (μM)	60	4.55	6.32	9.07
Amantadin (μM)	>100	21.39	0.78	>100
Rimantadin (μM)	>100	18.45	0.05	>100

[a] Required to cause a microscopically detectable alteration of normal cell morphology.

Table 8.9

Cytotoxicity and antiviral activity of compounds in HEL cell cultures, Corona viruses

	Feline Corona virus (FIPV)		Human Corona (SARS) virus	
	EC50 (μg/ml)	CC50 (μg/ml)	EC50 (μg/ml)	CC50 (μg/ml)
1	52 ± 19	>100	>100	>100
2	67 ± 47	>100	>100	>100
3	49 ± 17	>100	>100	>100
4	43 ± 2	>100	>100	>100
5	49 ± 10	⩾100	44 ± 10	>100
6	55 ± 19	>100	49 ± 21	>100
7	32 ± 1	>100	40 ± 1	>100
8	72 ± 40	>100	>100	>100
9	⩾100	>100	>100	>100
10	20 ± 21	>100	>100	>100
11	44 ± 5	>100	56 ± 13	>100
12	7.8 ± 8.0	81 ± 13	>100	>100
13	63 ± 32	>100	>100	>100

Table 8.10

Cytotoxicity and antiviral activity of compounds in HEL cell cultures, Herpes and Vaccinia viruses. Tannins added after virus administration

Cytotoxicity and antiviral activity of compounds in HEL cell cultures						
Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μg/ml)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus^{-1} TK^{-} KOS ACV^{r}
1	200	6 ± 2	1.8 ± 0.2	>40	>40	6 ± 2
2	200	4 ± 0	2 ± 1	8 ± 0	>40	4 ± 0
3	200	15 ± 1	3 ± 1	24 ± 1	>40	17 ± 3
4	200	15 ± 1	8 ± 0	24 ± 1	>40	20 ± 2
5	⩾40	>40	>40	20 ± 2	>40	>40
6	⩾40	>40	8 ± 0	>40	>40	>40
7	⩾40	8 ± 0	>40	20 ± 2	>40	>40
8	40	4 ± 0	4 ± 0	8 ± 0	>8	4 ± 0
9	⩾40	>40	>40	>40	>40	>40
10	40	8 ± 0	4 ± 0	8 ± 0	>8	>8
11	40	>8	8 ± 0	>8	>8	>8
12	40	>8	8 ± 0	>8	>8	>8
13	200	>40	>40	>40	>40	>40
Brivudin (μM)[c]	>250	0.016	10	6	>250	50
Ribavirin (μ)[c]	>250	250	50	30	50	250
Acyclovir (μM)[c]	>250	0.08	0.08	>250	>250	50
Ganciclovir (μM)[c]	>100	0.0064	0.032	>100	>100	12
Compounds added prior to virus administration						

[a] Required to cause a microscopically detectable alteration of normal cell morphology.

[b] Required to reduce virus-induced cytopathogenicity by 50%.

[c] Controls.

Table 8.11

Cytotoxicity and antiviral activity of compounds in Vero cell cultures, influenza viruses

Cytotoxicity and antiviral activity of compounds in Vero cell cultures						
Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μg/ml)				
		Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
1	400	>80	>80	>80	>80	>80
2	>400	>400	>400	149.5 ± 24	149.5 ± 24	169.5 ± 13
3	400	>80	>80	>80	>80	>80
4	400	>80	>80	>80	>80	>80
5	400	>80	>80	>80	>80	>80
6	400	>80	>80	>80	>80	>80
7	80	>40	>40	>40	>40	>40
8	80	>40	>40	>40	>40	>40
9	400	>80	>80	>80	>80	>80
10	400	>80	>80	>80	>80	>80
11	400	>80	>80	>80	>80	>80
12	400	>80	>80	80	>80	>80
13	80	>16	>16	>16	>16	>16
Brivudin[c]	>400	>400	>400	>400	>400	>400
(S)-DHPA[c]	>400	>400	>400	>400	>400	>400
Ribavirin[c]	>400	240	80	240	>400	240

[a] Required to cause a microscopically detectable alteration of normal cell morphology.

[b] Required to reduce virus-induced cytopathogenicity by 50%.

[c] Controls.

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