MALDI-ToF Analysis of Tannin-Resorcinol Resins by Alternative Aldehydes: Glyoxal and Glutaraldehyde

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ABSTRACT: Glyoxal and glutaraldehyde are two viable alternatives to formaldehyde for the preparation of tannin-resorcinol-aldehyde adhesive but lead to less resistant glue joint. Tannin-resorcinol-glyoxal (TRG1) and tannin-resorcinol-glutaraldehyde (TRG2) resins have been prepared and analyzed by matrix-assisted laser desorption/ionization time of flight (MALDI-ToF) spectrometry to understand the chemical process behind the pre-curing of these resins and possibly the causes of this lower resistance. The analysis showed that TRG resins are not a simple mix of resorcinol-aldehydes oligomers and flavonoids, but a much more complex combination of various species including tannin-aldehydes and tannin-resorcinol oligomers.

KEYWORDS: Tannin resin, natural resin, MALDI-ToF

1 INTRODUCTION

Adhesives based on resorcinol and formaldehyde are commonly used for wood bonding, especially in the fabrication of laminated wood beams, with or without the use of phenol to substitute part of the expensive resorcinol. They can harden at room temperature in several hours and give suitable resistance for structural applications, even in outdoor and moist conditions. It has been shown in the past that natural-based phenolic products, namely condensed tannins [1], could be used to substitute the phenol, thus creating even cheaper and bio-based adhesives, which have been used for decades in some countries in the Southern Hemisphere [2, 3]. The interest in the use of vegetable tannins as a natural phenolic compound has grown in the past few years in response to the foreseen upcoming scarcity of fossil resources. These adhesives have been tested in different fields than wood gluing, such as composite materials [4, 5] or preparation of foams [6]. The tannin-resorcinol-formaldehyde adhesive was originally developed quite empirically over the 1970s [2], and not much was known about the structure of the oligomers composing this wood adhesive until recently, when MALDI-ToF analysis was done in order to understand its precise chemical composition [7]. However, formaldehyde being considered as toxic, alternative nontoxic and nonvolatile aldehydes have been considered for preparing these adhesives [8], namely glyoxal and glutaraldehyde, with good wood bonding results. Considering the much lower reactivity of these two aldehydes, it is of interest to see what type of compounds are formed in the reaction of copolymerization leading to the formation of these resins that present lower properties than the standard TRF. The present study is then a MALDI-ToF investigation on the structure of the oligomers formed in the preparation of tannin-resorcinol-glyoxal and tannin-resorcinol-glutaraldehyde resins.

2 EXPERIMENTAL

2.1 Materials

The tannin extract used for the preparation of the resin was commercial mimosa tannin extract (non sulfited, hot water extracted) from Silvateam (San Michele Mondovi, Italy).

Resorcinol, glyoxal and glutaraldehyde were purchased from ACROS Organics (Geel, Belgium).

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Table 1 TRG formulations of resins.

Resin	Tannin	Resorcinol	Water	Methanol	Glyoxal	Glutaraldehyde	Sodium hydroxyde
TRG1	35,10	17,50	33,31	9,86	2,29	–	2,11
TRG2	35,10	17,50	32,59	9,86	–	2,29	2,11

Formaldehyde 37% in solution was purchased from Roth (Karlsruhe, Germany).

Sodium hydroxide 33% in solution was purchased from Carlo Erba Reagents (Val de Reuil, France).

Methanol was purchased from VWR Prolabo (Fontenay-sous-Bois, France).

2.2 Resin Preparation

The different formulations are described in Table 1. The resins were prepared as followed, based on the work of Pizzi and Roux [2]. First, water and methanol were mixed together in a glass balloon with cooling column using a mechanical stirrer and heated to 40°C with a water bath to ease the dissolution of tannins. Mimosa tannins powder was then added and dissolved in the solution. Resorcinol was added and dissolved. Aldehyde solution and sodium hydroxide were added, bringing the resin at pH8. The solution was brought to 70°C for 1 h with continuous stirring and then cooled down. The solution was stored in hermetic containers. The pH of 8 was chosen for process reasons: at higher pH where the phenols are more reactive the gelation occurred within seconds, making the glue almost unusable [4]. At low pH the opposite behavior is observed: the hardening of the TRF is too slow, thus the glue is not suitable for composite making at high temperature or for wood gluing at room temperature. At pH 8 the glue has suitable strength, while still being conservable long enough to be able to work, with a pot-life of at least 3 hours.

2.3 MALDI-ToF Analysis

The dry droplet sample preparation method was used. The TRF samples were dissolved (5 mg/ml) in a solution made of one part of 1:1 water:acetone solution and one part of NaCl solution at 0.1 mol/L in water, the NaCl being used for the enhancement of ion formation. The sample solutions were left at room temperature (20°C) for 24 h. Then these sample solutions were mixed with 2,5-dihydroxy benzoic acid in 1:1 volume proportion and 1.5 µl of the resulting solution mix was placed onto the MALDI target; only after that was NaCl matrix added onto the target support plate (0.1 mol/L in water) for the enhancement of ion formation. After evaporation of the solvent, the MALDI target was introduced into the spectrometer. Red phosphorus was used for calibration.

The spectra were recorded on a Shimazdu AXIMA Performance instrument. The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The length of a single laser pulse was 3 ns. The measurements were carried out using the following conditions: polarity-positive, flight path-linear, mass-high (20 kV acceleration voltage), and 1000 pulses per spectrum. The delayed extraction technique was used by applying delay times of 200–800 ns. A second analysis has been performed for both resins in order to confirm the presence of the peaks that were observed. The spectra were then recorded without any smoothing and the intensities of the peaks were obtained by direct reading.

3 RESULTS AND DISCUSSION

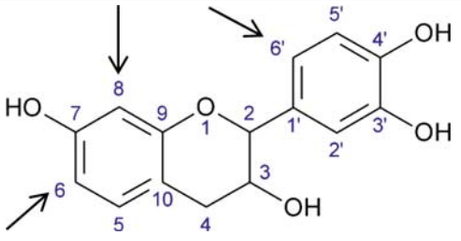
3.1 Molar Masses and Peak Reading

Initial components of the glues:

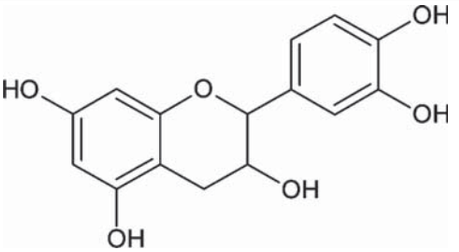
- Glyoxal = 58Da.
- Glutaraldehyde = 100Da.
- Resorcinol = 110Da.
- Flavonoid monomers = 274 / 290 / 306Da.

There are 4 main flavonoid units in mimosa tannin: fisetinidin, catechin, robinetinidin and gallocatechin. The ones in higher proportions are robinetinidin and fisetinidin, about 70% and 25% respectively [9]. Catechin and gallocatechin represent most of the last 15%. However, it is impossible to distinguish a catechin monomer from a robinetinidin monomer on a MALDI-ToF spectrum, these two flavonoids having the same molecular formula; the only difference is that the placement of one hydroxyl group is on C5′ for robinetinidin and on C5 for catechin. In Table 2, the phenol rings and the carbon numbers are labeled on the fisetinidin, starting with the number 1 being the oxygen atom of the ether in the heterocyclic ring. The arrows indicate the reactive sites of the flavonoids. The C6 and the C8 are the most reactive ones, C8 higher than C6 for catechin and gallocatechin (the activation of carbons being better on the position para of the

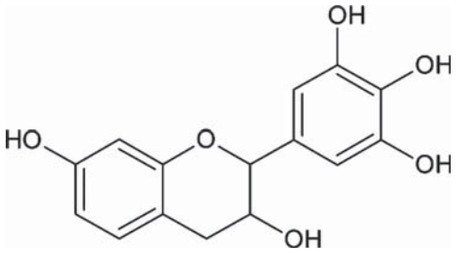
Table 2 Flavonoid monomers and their active sites.



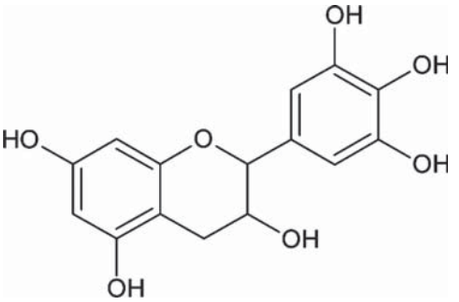
Fisetinidin (274Da)



Catechin (290Da)



Robinetinidin (290Da)



Gallocatechin (306Da)

hydroxyl groups than on ortho) and C6 higher than C8 for fisetinidin and robinetinidin. The C6′ carbon of the B-ring can also react, but only under very alkaline conditions (pH ≥ 10), which is not the case in the present study, as the resins were prepared at p H8.

Fisetinidin will then be referred to as "A-unit," robinetinidin and catechin as "B-unit" (these two monomers being indistinguishable by their molar masses) and gallocatechin as "C-unit."

To interpret the MALDI-ToF spectra, the components to consider are:

- Ethylene unit = 28Da (glyoxal without the 2 aldehyde groups: $–(CH_2)_2–$).
- Pentamethylene unit = 70Da (glutaraldehyde without the 2 aldehyde groups: $–(CH_2)_5–$).
- Resorcinol unit = 108Da (110 – 2Da for the 2 hydrogen atoms removed for bonding).
- Flavonoid monomer unit = 272 / 288 / 304Da (274 / 290 / 306 – 2Da for the 2 hydrogen atoms removed for bonding).

These masses correspond to the molecules with carbocation terminal carbons. These carbons could be of different forms, thus involving different masses (see below).

- Na marker = 23Da.
- DHB = 154Da (2,5-dihydroxybenzoic acid).

The terminal carbons for these components could be of different forms:

1. as a carbocation C^+ = 0Da ($–CH_2^+$)
2. with a "–H end" = 1Da ($–CH_3$)
3. with a "–OH end" (on glyoxal and glutaraldehyde units only) = 17Da ($–CH_2OH$)

The combination formula would then be:

$$a*272 + b*288 + c*304 + d*28 + r*108 + t2*1 + t3*17 + 23 \quad \text{(TRG1)}$$

$$a*272 + b*288 + c*304 + d*70 + r*108 + t2*1 + t3*17 + 23 \quad \text{(TRG2)}$$

a = number of A-unit,
b = number of B-unit,
c = number of C-unit,
d = number of aldehyde-based unit (ethylene or pentamethylene),
r = number of resorcinol unit,
t2 = number of "–H end" terminal group,
t3 = number of "-OH end" terminal group,
23 = mass of the Na marker.

Example: A peak at 340Da could be read as a combination of 2 resorcinol units and 3 ethylene groups with a carbocation $–CH_2^+$ at one end and an alcohol group at the other end (Figure 1).

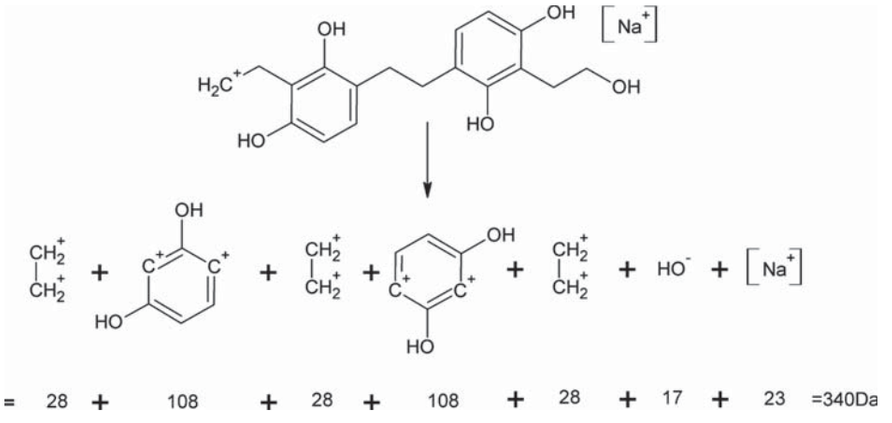


Figure 1 Example of combination for the 340Da peak.

3.2 Flavonoid Oligomers

Flavonoids are present in tannin as polyflavonoid (2 to 11 units), the monomers being linked by C4-C6 or C4-C8 bonds. Thus, we have profisetinidin (fisetinidin oligomers), prorobinetinidin (robinetinidin oligomers), procyanidin (catechin and epicatechin oligomers) and prodelphinidin (gallocatechin and epigallocatechin oligomers). Monomers are in general always present [10]. Dimers, trimers, tetramers and pentamers can be identified on the spectra: 567–633Da, 841–935Da and 1113–1161Da ranges in Table 3 or 565–633Da, 874–935Da, 1111–1178Da and 1401–1476Da ranges in Table 4, as some of the monoflavonoids (331Da). The most present seems to be the flavonoids dimers as the intensity of the 565–633Da peaks indicate (Figure 2 and Figure 3). These observations are in line with the low average degree of polymerization of the mimosa tannins, which is 4.9 [11, 12]. These unreacted flavonoids with a degree of polymerization up to 3 were also found in TRF resin in similar proportions, while tetramers and pentamers were not observed [7].

3.3 Resorcinol–Aldehydes Chain Polymerization

Reaction: Glyoxal and glutaraldehyde react by condensation with resorcinol preferentially on its C4 and C6 carbons, but also on C2, and lead to an n-hydroxyethyl or n-hydroxypentyl group. Another resorcinol then reacts on the C1 of this alcohol, and so on, creating a resorcinol/glyoxal or resorcinol/glutaraldehyde chain (Figure 4 and Figure 5). In solution, aldehydes are present in the form of alcohol (e.g., formaldehyde as dihydroxymethane). In the following equations, all reacted aldehydes will be presented this way.

The intensities of the peaks from both resins indicate that these linear polymers are present in significant quantities. For TRG1 most of the oligomers included 4 resorcinol units: 537–545Da range at an intensity of 36 to 72% with 3 ethylene bridges and 567Da at 14% with a fourth ethylene unit or 582Da at 8% if this fourth unit bears an alcohol terminal group. Other peaks of high intensities could be seen for oligomers with 5 resorcinol units (707Da at 9%, 721Da at 17%, 730Da at 11%), 3 resorcinol units (493Da at 15%) or 2 resorcinol units (407Da at 12%), but the rest are under 8%.

For TRG2 these peaks are less important, except for the 551Da peak: 2 resorcinol and 4 pentamethylene units, which is the 100% reference. Then the highest are also for the oligomers including 4 resorcinol units: 669Da at 22% with 3 pentamethylene bridges, 737Da at 8% with a fourth pentamethylene unit and 821Da at 16% with a fifth pentamethylene unit. Oligomers with 5 or more resorcinol units have peaks under 6%, and only oligomers with 3 or 2 resorcinol units are remarkable with 663Da at 13% with one pentamethylene bridge and 491Da at 14% with 4 pentamethylene units respectively.

Just as for tannin-resorcinol-formaldehyde resin [7], in both TRG formulations it is possible to see oligomers with the alkane chains derived from the aldehydes that present $–CH_2^+$ and/or $–CH_2OH$ reactive groups. The polymer mix is therefore suitable for further reticulation reactions using these resorcinol-aldehydes oligomers. The main difference between the resorcinol-formaldehyde oligomers from the TRF and the resorcinol-glyoxal or resorcinol-glutaraldehyde oligomers is their molecular weight, due to the longer carbon chain of these two aldehydes. Oligomers containing 2 to 4 resorcinol units ranged from 274 to 429Da in TRF, 407 to 567Da in TRG1 and 491 to 837Da

Table 3 Experimental data peaks, relative percentages and interpretations of the MALDI-ToF spectra of TRG1: A/B/C = flavonoids, R = resorcinol, Gly/Gly+/GlyOH = glyoxal. Oligomers with flavonoid units are show in bold face.

Range	Peak relative intensity	Interpretations
177–178	13–14%	DHB
331	4%	**C**
340 / **356** / **373**	1% / 2% / 1%	**A**-*GlyOH* / **B**-*GlyOH* / **C**-*GlyOH*
407	12%	R-*Gly*-R-*Gly*-R
407–409 / **423** / **438**	12–18% / 4% / 5%	**A**-R / **B**-R / **C**-R
430 / **449** / **465**	7% / 5% / 4%	**A**-*Gly*-R / **B**-*Gly*-R / **C**-*Gly*-R
460 / **475** / **493**	4% / 7% / 15%	+*Gly*-**A**-*Gly*-R / +*Gly*-**B**-*Gly*-R / +*Gly*-**C**-*Gly*-R
493	15%	*HOGly*-R-*Gly*-R-*Gly*-R-*GlyOH*
537–545	72–36%	R-*Gly*-R-*Gly*-R-*Gly*-R
554	**100%**	*HOGly*-**C**-Gly-R-*GlyOH*
567	14%	+*Gly*-R-*Gly*-R-*Gly*-R-*Gly*-R
567 / **582** / **603** / **620–621** / **633**	14% / 8% / 3% / 26–16% / 4%	**AA** / **AB** / **AC** or **BB** / **BC** / **CC**
567 / **582**	14% / 8%	R-*Gly*-**A**-*Gly*-R / R-*Gly*-**B**-*Gly*-R
647 / **664**	26% / 10%	**B**-*Gly*-**C** / **C**-*Gly*-**C**
677 / **695** / **713** / **728** / **742**	4% / 4% / 20% / 16% / 7%	**AA**-R / **AB**-R / **AC**-R or **BB**-R / **BC**-R / **CC**-R
707 / **721** / **737** / **752** / **768**	9% / 17% / 5% / 5% / 3%	**AA**-*Gly*-R / **AB**-*Gly*-R / **AC**-*Gly*-R or **BB**-*Gly*-R / **BC**-*Gly*-R / **CC**-*Gly*-R
721	17%	R-*Gly*-R-*Gly*-R-*Gly*-R-*Gly*-R-*GlyOH*
721	17%	**B**-*Glu*-R-*Glu*-R-*Glu*-R
730	11%	HOGly-R-*Gly*-R-*Gly*-R-*Gly*-R-*Gly*-R-*GlyOH*
768	3%	+*Glu*-R-*Glu*-R-*Glu*-R-*Glu*-R-*Glu*-R-*Glu*-R-*Glu*+
823 / **837** / **850**	9% / 2% / 9%	R-**AC**-R or R-**BB**-R / R-**BC**-R / R-**CC**-R
841 / **857** / **874–875** / **889** / **903–904** / **921** / **935**	2% / 2% / 6–8% / 7% / 6–5% / 3% / 4%	**AAA** or **AAB** or **AAC** or **ABB** / **ABC** or **BBB** / **ACC** or **BBC** / **BCC** / **CCC**
884 / **903** / **918** / **935** / **950** / **966**	6% / 6% / 5% / 4% / 2% / 2%	**AAA**-*GlyOH* / **AAB**-*GlyOH* / **AAC**-*GlyOH* or **ABB**-*GlyOH* / **ABC**-*GlyOH* or **BBB**-*GlyOH* / **ACC**-*GlyOH* or **BBC**-*GlyOH* / **BBC**-*GlyOH*
884 / **903** / **918** / **935** / **950** / **966**	6% / 6% / 5% / 4% / 2% / 2%	**AA**-*Gly*-**B** / **AA**-*Gly*-**C** or **AB**-*Gly*-**B** / **AB**-*Gly*-**C** or **BB**-*Gly*-**B** / **AC**-*Gly*-**C** or **BB**-*Gly*-**C** / **BC**-*Gly*-**C** / **CC**-*Gly*-**C**
903	6%	*HOGly*-R-*Gly*-R-*Gly*-R-*Gly*-R-*Gly*-R-*Gly*-R-*Gly*-R-*GlyOH*
1038	4%	**ACC**-*Gly*-R or **BBC**-*Gly*-R
1113 / **1129** / **1145** / **1161**	2% / 2% / 1% / 1%	**AAAA** / **AAAB** / **AAAC** or **AABB** / **AABC** or **ABBB**

Table 4 Experimental data peaks, relative percentages and interpretations of the MALDI-ToF spectra of TRG2: A/B/C = flavonoids, R = resorcinol, Glu/Glu+/GluOH = glutaraldehyde. Oligomers with flavonoid units are show in bold face.

Range	Peak relative intensity	Interpretations
178	6%	DHB
331	2%	**C**
383 / **400** / **415**	2% / 8% / 3%	**A**-*GluOH* / **B**-*GluOH* / **C**-*GluOH*
407 / **441**	8% / 7%	**A**-R / **C**-R
475 / **491** / **507**	4% / 14% / 5%	**A**-*Glu*-R / **B**-*Glu*-R / **C**-*Glu*-R
491	14%	R-*Glu*-R-*Glu*-R
551	**100%**	$(HOGlu)_2$-R-Glu-R-*Glu*+
565 / **580–590** / **604** / **620** / **633**	21% / 10–23% / 6% / 44% / 13%	**AA** / **AB** / **AC** or **BB** / **BC** / **CC**
545 / **555–565** / **580**	33% / 89–21% / 10%	+*Glu*-**A**-*Glu*-R / +*Glu*-**B**-*Glu*-R / +*Glu*-**C**-*Glu*-R
640 / **655** / **669** / **685** / **703**	13% / 3% / 22% / 20% / 8%	**A**-*Glu*-**A** / **A**-*Glu*-**B** / **A**-*Glu*-**C** or **B**-*Glu*-**B** / **B**-*Glu*-**C** / **C**-*Glu*-**C**
655 / **669** / **685**	3% / 22% / 20%	R-*Glu*-**A**-*Glu*-R / R-*Glu*-**B**-*Glu*-R / R-*Glu*-**C**-*Glu*-R
663	13%	*HOGlu*-R-*Glu*-R-*Glu*-R-*GluOH*
669	22%	R-*Glu*-R-*Glu*-R-*Glu*-R
691 / **712** / **727** / **743**	12% / 31% / 33% / 13%	**AB**-R / **AC**-R or **BB**-R / **BC**-R / **CC**-R
737	8%	+*Glu*-R-*Glu*-R-*Glu*-R-*Glu*-R
743 / **764** / **780** / **793**	13% / 4% / 4% / 8%	**AA**-*Glu*-R / **AB**-*Glu*-R / **AC**-*Glu*-R or **BB**-*Glu*-R / **BC**-*Glu*-R
821 / **837**	16% / 3%	R-**AC**-R or R-**BB**-R / R-**BC**-R
821	16%	+*Glu*-R-*Glu*-R-*Glu*-R-*Glu*-R-*GluOH*
837	3%	*HOGlu*-R-*Glu*-R-*Glu*-R-*Glu*-R-*GluOH*
874 / **889** / **903** / **919–924** / **935**	8% / 13% / 14% / 8–6% / 5%	**AAC** or **ABB** / **ABC** or **BBB** / **ACC** or **BBC** / **BCC** / **CCC**
924 / **942** / **955** / **972** / **995**	6% / 6% / 6% / 3% / 4%	**AAA**-*GluOH* / **AAB**-*GluOH* / **AAC**-*GluOH* or **ABB**-*GluOH* / **ABC**-*GluOH* or **BBB**-*GluOH* / **ACC**-*GluOH* or **BBC**-*GluOH*
911 / **924** / **942** / **955** / **972** / **995**	5% / 6% / 6% / 6% / 3% / 4%	**AA**-*Glu*-**A** / **AA**-*Glu*-**B** / **AA**-*Glu*-**C** or **AB**-*Glu*-**B** / **AB**-*Glu*-**C** or **BB**-*Glu*-**B** / **AC**-*Glu*-**C** or **BB**-*Glu*-**C** / **BC**-*Glu*-**C**
935	5%	**A**-*Glu*-R-*Glu*-R-*Glu*-R-*GluOH*
1024	4%	R-*Glu*-R-*Glu*-R-*Glu*-R-*Glu*-R-*Glu*-R
1051 / **1065** / **1079** / **1096**	5% / 7% / 7% / 6%	**AAC**-*Glu*-R or **ABB**-*Glu*-R / **ABC**-*Glu*-R or **BBB**-*Glu*-R / **ACC**-*Glu*-R or **BBC**-*Glu*-R / **BCC**-*Glu*-R
1079 / **1096**	7% / 6%	**A**-*Glu*-R-*Glu*-R-*Glu*-R-*Glu*-R-*Glu*+ / **B**-*Glu*-*R*-*Glu*-*R*-*Glu*-*R*-*Glu*-*R*-*Glu*+
1111 / **1132** / **1178**	4% / 3% / 6%	**AAAA** / **AAAB** / **AACC** or **ABBC** or **BBBB**
1401 / **1422** / **1449** / **1476**	2% / 5% / 6% / 5%	**AAAAB** / **AAAAC** or **AAABB** / **AAACC** or **AABBC** or **ABBBC** / **AACCC** or **ABBCC** or **BBBBC**
1178	6%	+*Glu*-R-*Glu*-R-*Glu*-R-*Glu*-R-*Glu*-R-*Glu*-R-*Glu*-R-*GluOH*

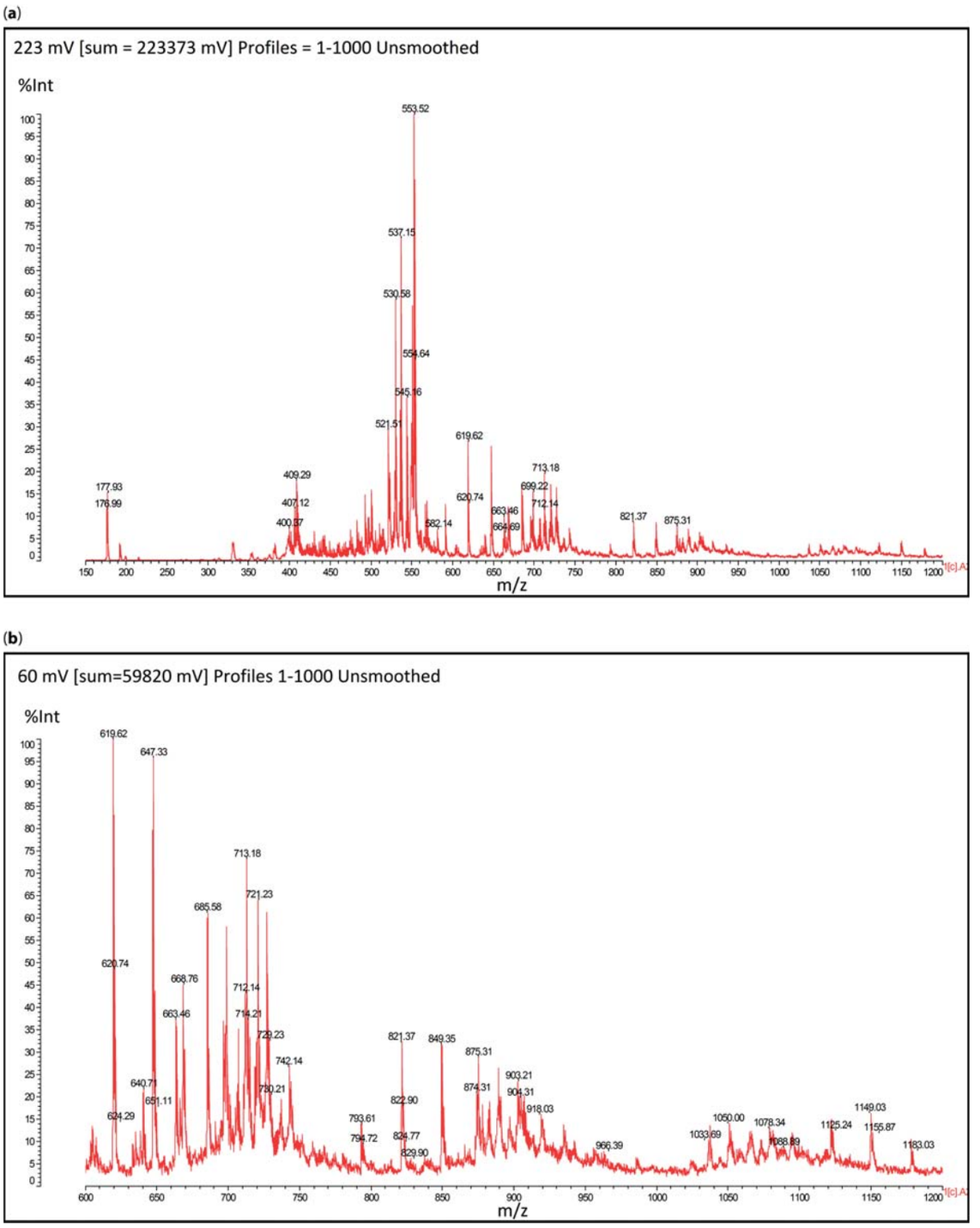


Figure 2 MALDI-ToF spectra of TRG1 resin prepared at pH 8; **(a)** 150–1200Da range and **(b)** detail of the 600–1200Da range.

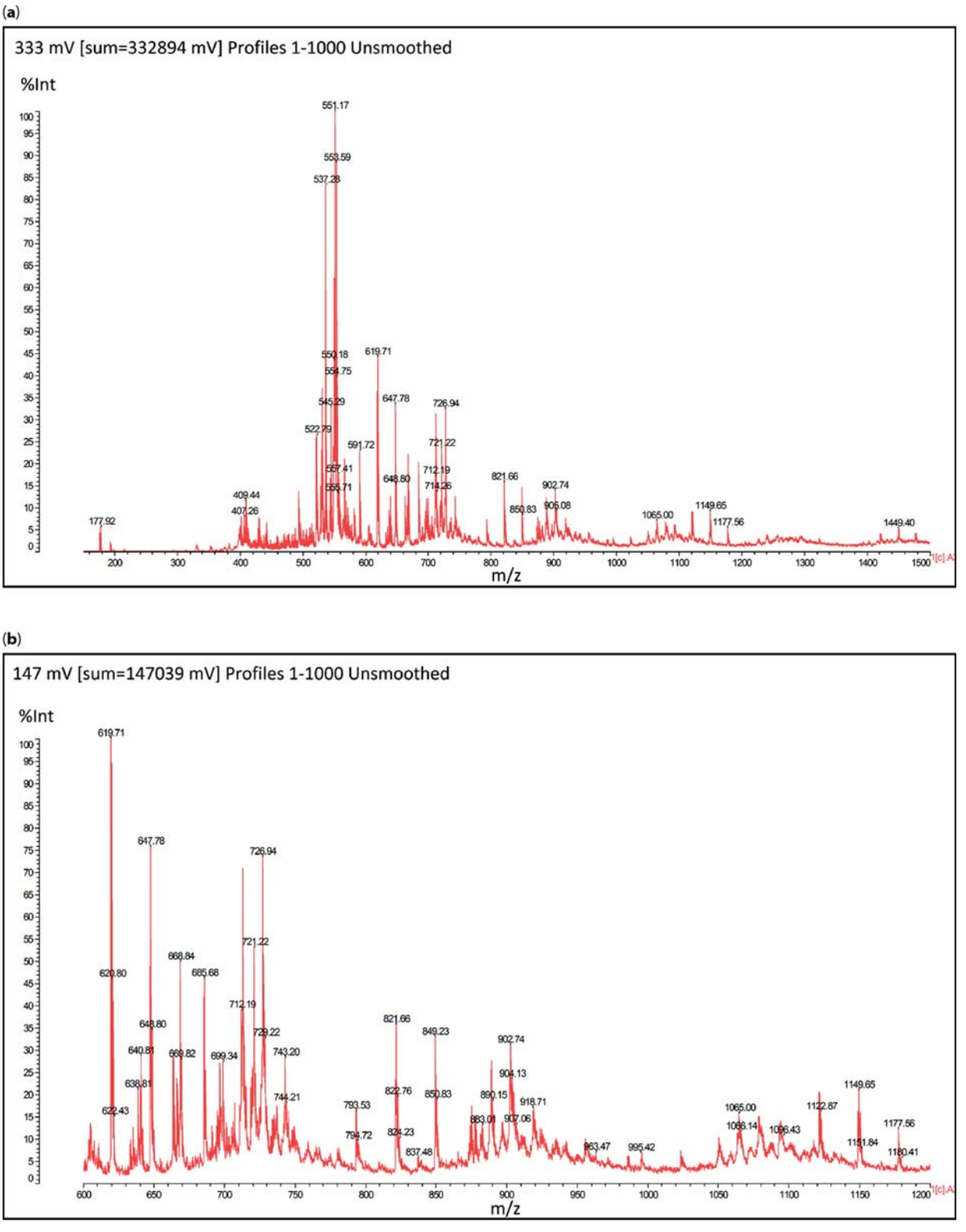


Figure 3 MALDI-ToF spectra of TRG2 resin prepared at pH 8; **(a)** 150–1500Da range and **(b)** detail of the 600–1200Da range.

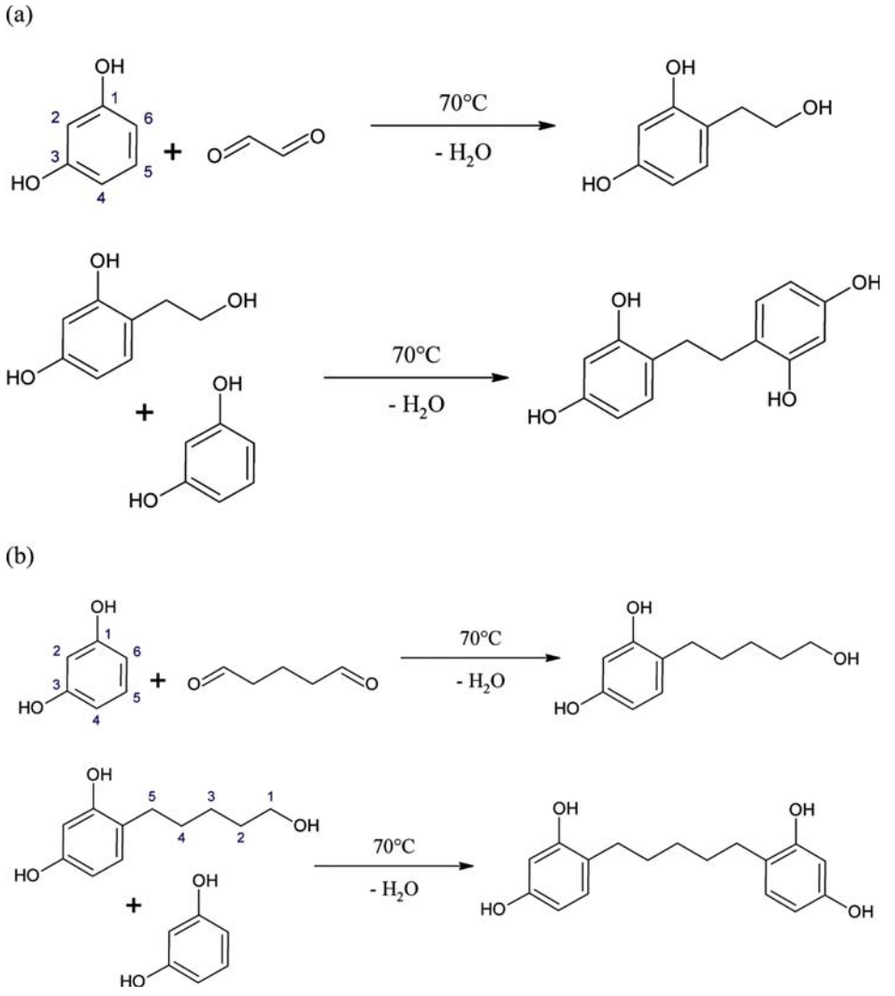


Figure 4 **(a)** Resorcinol/glyoxal and **(b)** resorcinol/glutaraldehyde reactions.

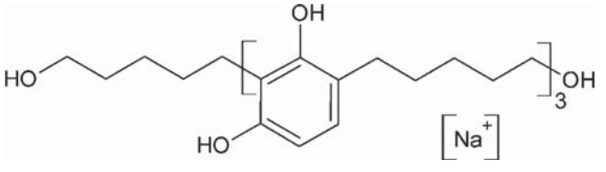


Figure 5 Resorcinol/glutaraldehyde linear polymer of 661Da (peak read at 663Da).

in TRG2. Oligomers with 5 or 6 resorcinol units which are not present in TRF could be found in both TRG1 (721 to 903Da range) and TRG2 (669 to 1024Da range).

Example: The peak at 663Da on Figure 3. There are 3 resorcinol units and 4 glutaraldehyde units with 2 –OH terminal groups: 3*108 (resorcinol) + 4*70 (glutaraldehyde) + 2*17 (-OH group) + 23 (Na) = 661Da.

3.4 Tannin Reaction with Aldehydes and Polymerization by Ethylene or Pentamethylene Bridges

Reaction: The glyoxal (TRG1) or glutaraldehyde (TRG2) is linked by its terminal carbon to the C8 or C6 of the flavonoid. An n-hydroxyethyl or n-hydroxypentyl group is formed. Another flavonoid can then react on the C1 of this n-hydroxyethyl or n-hydroxypentyl (Figure 6 and Figure 8).

There are several peaks that show the intermediates formed at the end of the first step of the reaction. In TRG1 spectra there are peaks of flavonoids dimers with one hydroxyethyl unit ranging from 647 to 677Da with intensities up to 26%, or flavonoids trimers with one hydroxyethyl unit ranging from 884 to 966Da but with much lower intensities, between 2 and 7%. The

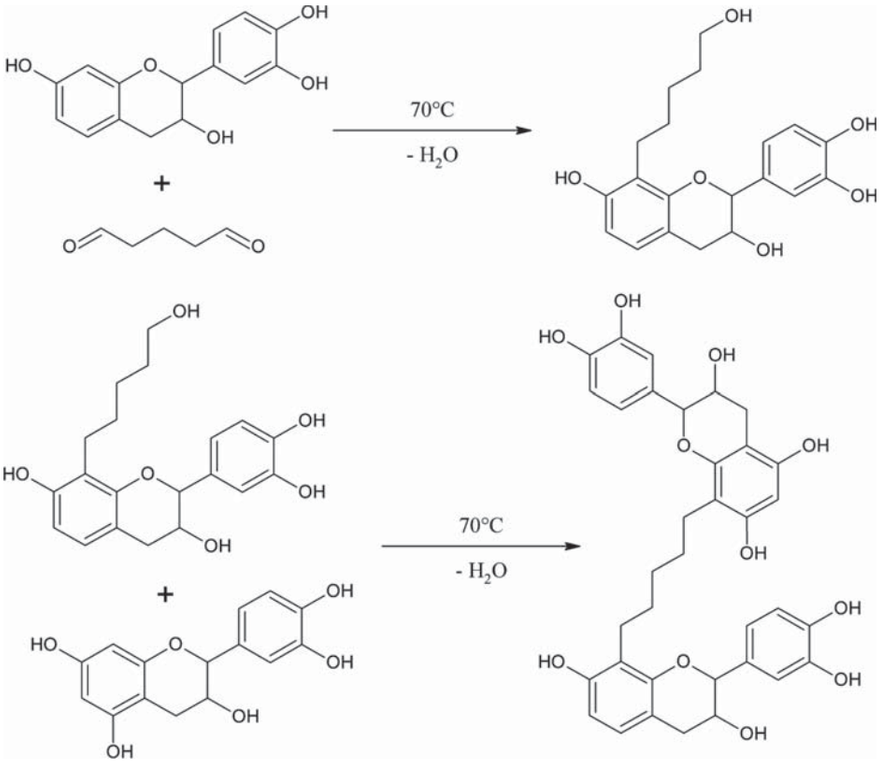


Figure 6 Flavonoid polymerization by a pentamethylene bridge.

peaks of hydroxyethyl unit with a flavonoid monomer are also present but at very low intensities: 340, 355 and 373Da, all at less than 2%.

But these peaks could also correspond to a different structure: 2 flavonoid monomers linked by an ethylene bridge in the case of the 647 to 677Da range or a flavonoid dimer linked to a flavonoid monomer in the same way for the 884 to 966Da range. Unfortunately these two possibilities cannot be told apart with the MALDI-ToF spectra and the peak could be a superposition of the two oligomers. However, these peaks clearly indicate that glyoxal has reacted with tannin oligomers during resin curing.

The same occurs with the TRG2 spectra: there are peaks of flavonoid dimers with one hydroxypentyl unit ranging from 685 to 720Da with intensities up to 31%, or flavonoid trimers with one hydroxypentamethylene unit ranging from 924 to 995Da, but again with much lower intensity, between 3 and 6%.

In this case, however, the peaks representing one hydroxypentyl unit with a flavonoid monomer are more visible: 383 to 416Da range at 2 to 8%, 8% being the most abundant B-unit. Thus, it seems that glutaraldehyde is more easily grafted onto the flavonoids than glyoxal. This is in line with previous experiments where TRG2 leads to better wood joints than TRG1 [8], results that could be explained by this aldehyde reacting better with tannin than glyoxal.

Again the hypothesis of flavonoid oligomers linked by a pentamethylene bridge is possible, having their corresponding peaks blended with the previous ones, but in both cases it shows that glutaraldehyde reacted with tannins.

It is also possible that two glyoxal or two glutaraldehyde units react with each other by aldol condensation (Figure 7), this reaction occurring preferentially under alkaline condition [13]. This is a rapid and reversible reaction.

These intermediate products, one or two aldehydes attached to flavonoids, were not observed in the case of TRF resin. This may be due to the fact that the formaldehyde being highly reactive quickly polymerized with resorcinol, which is slightly more reactive than the flavonoids [14]. Furthermore, the reaction was performed at pH 8, which is a pH of high reactivity of the phenols [2]. However no glyoxal nor glutaraldehyde monomers were observed on the spectra (see Tables 3 and 4), meaning that despite

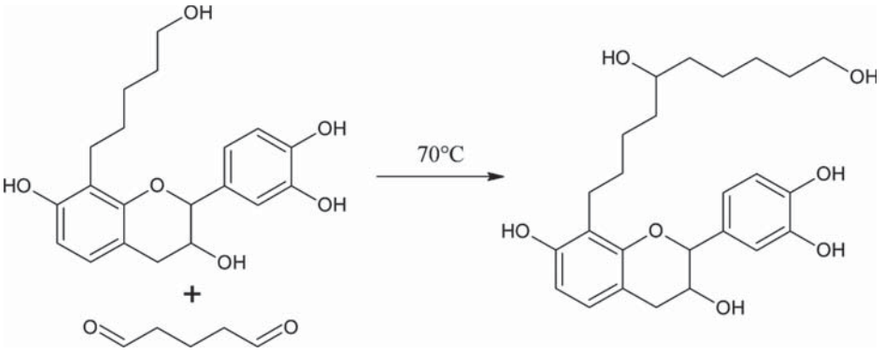


Figure 7 Formation of an ether link between two glutaraldehyde units.

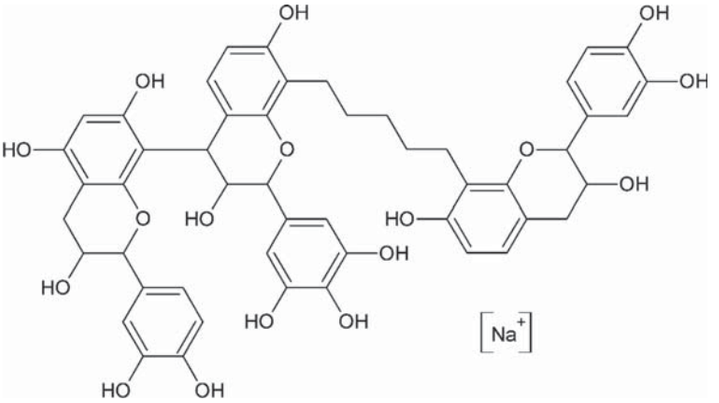


Figure 8 Flavonoid/glutaraldehyde branched polymer of 943Da (peak read at 942Da).

their lower reactivity all aldehydes were bonded to the phenolic products.

Example: The peak at 942Da on Figure 3. One AB-unit and one B-unit linked by a pentamethylene bridge: 272 + 2*288 (flavonoids) + 70 (glutaraldehyde) + 2*1 (-H end) + 23 (Na) = 943Da.

3.5 Direct Addition of Resorcinol onto Flavonoids

Reaction: It has been shown in previous studies [15, 16] that the direct addition of resorcinol on flavonoids was possible on the C2 or C4 sites of the heterocyclic ring in a reaction involving the opening of the heterocyclic ring followed by the formation of a carbocation on the C2 carbon, on which the resorcinol can react (Figure 9 and Figure 10).

Different peaks that correspond to the products of this reaction could be found in both TRG1 and TRG2 spectra. The 407 to 438Da range at 4 to 18% in TRG1 and the 407 to 441Da range at 8 to 7% in TRG2 correspond to a resorcinol unit grafted on a flavonoid monomer. For the flavonoids dimers it is the 677 to 742Da range at 3 to 26% in TRG1 and 691 to 743Da range at 4 to 13% in TRG2. Trimers cannot be seen except for a couple of very small peaks (maximum 2%) at 950 and 966Da in the TRG1 spectra.

Even if some of these peaks are once more superimposed on other signals, they give the information that some of these reaction products are present in the TRG resins. They were not observed in the case of TRF resin [7]. This is due to the fact that resorcinol interacts preferentially with the highly reactive formaldehyde, preventing the formation of these flavonoid-resorcinol oligomers.

Example: The peak at 727Da on Figure 2 and Figure 3. One BC-unit oligomer and one resorcinol unit: 288 + 304 (flavonoids) + 108 (resorcinol) + 2*1 (-H end) + 2 (cycle opening) + 23 (Na) = 727Da.

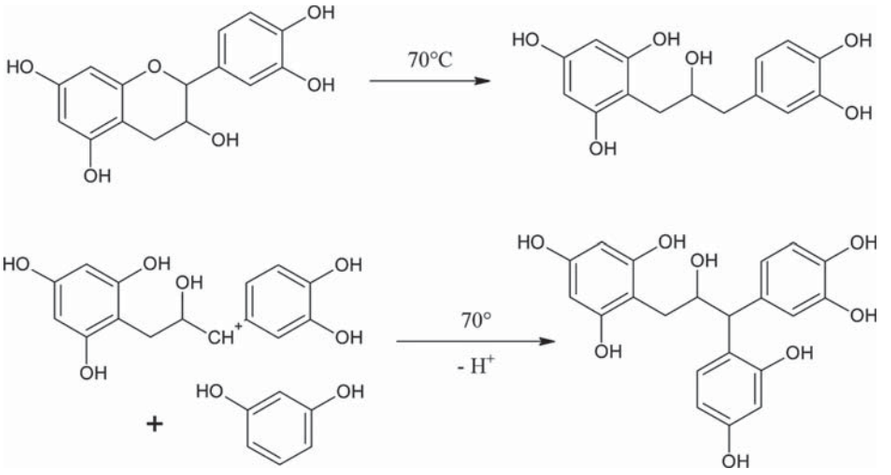


Figure 9 Reaction of resorcinol on the C2 of a flavonoid unit.

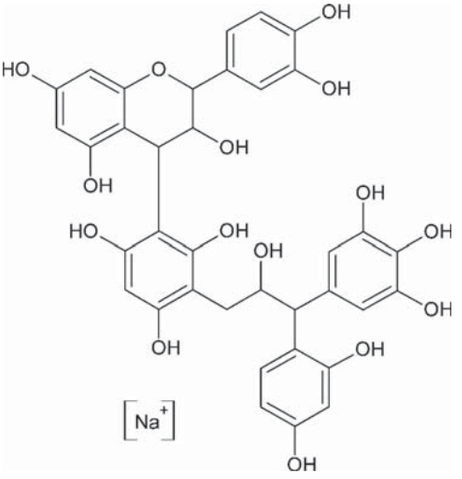


Figure 10 Flavonoid/resorcinol polymer of 727Da (peak read at 727Da).

3.6 TRG1: Tannin–Glyoxal–Resorcinol

Reaction: Glyoxal reacts with flavonoids preferentially on the C8 site, then on C6 or C4. An n-hydroxyethyl group is formed, which resorcinol can react with on the n-hydroxyethyls C1 (Figure 11). Resorcinol–glyoxal chains that still possess reactive groups can also react with polyflavonoids (Figure 12 and Figure 13).

The related peaks could be seen at various intensities corresponding to different combinations. Flavonoid dimers with one ethylene unit and one resorcinol unit are the most important, ranging from 709 to 768Da at 5 to 17%, 17% being the AB dimer, namely one robinetinidin and one fisetinidin. TRG1 based on trimers could hardly be seen, with a single corresponding peak at 1038Da at the intensity of 4%. The TRG1 with flavonoid monomers is a little more visible, ranging from 430 to 465Da at intensities from 4 to 7%.

It means that these fully reacted TRG units are less present than the oligomeric intermediates, namely tannin linked only to glyoxal or to resorcinol.

Example: The peak at 721Da on Figure 2. One AB-unit oligomer, one glyoxal unit and one resorcinol unit: 272 + 288 (flavonoids) + 26 (glutaraldehyde) + 108 (resorcinol) + 2*1 (-H end) + 23 (Na) = 719Da.

3.7 TRG2: Tannin–Glutaraldehyde–Resorcinol

Reaction: Glutaraldehyde reacts with flavonoids preferentially on the carbon at C8, then on C6 or C4. An n-hydroxypentyl group is formed, which can react with resorcinol on the n-hydroxypentyls C1 (Figure 14).

Resorcinol–glutaraldehyde chains that still possess reactive groups can also react with polyflavonoids (Figure 15 and Figure 16).

The TRG2 based on either flavonoids monomers, dimers and trimers could be seen at similar intensities: 475 to 507Da at 4 to 14% for the monomers, the 14% peak being the one corresponding to the B-unit flavonoid, and 743 to 793Da at 4 to 13% for the dimers. Surprisingly, the highest 13% peak is the one for the AA flavonoid dimer made of fisetinidins, which are supposed to be less present than robinetinidins [9]. It

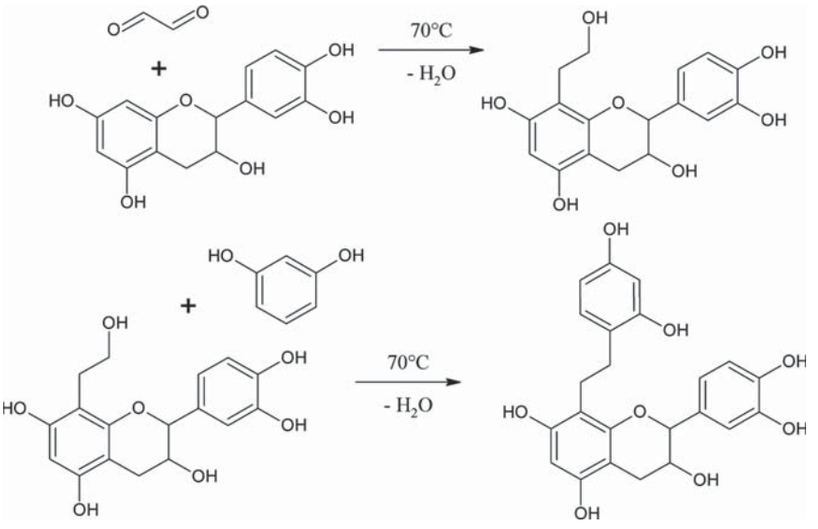


Figure 11 Tannin/glyoxal/resorcinol reaction.

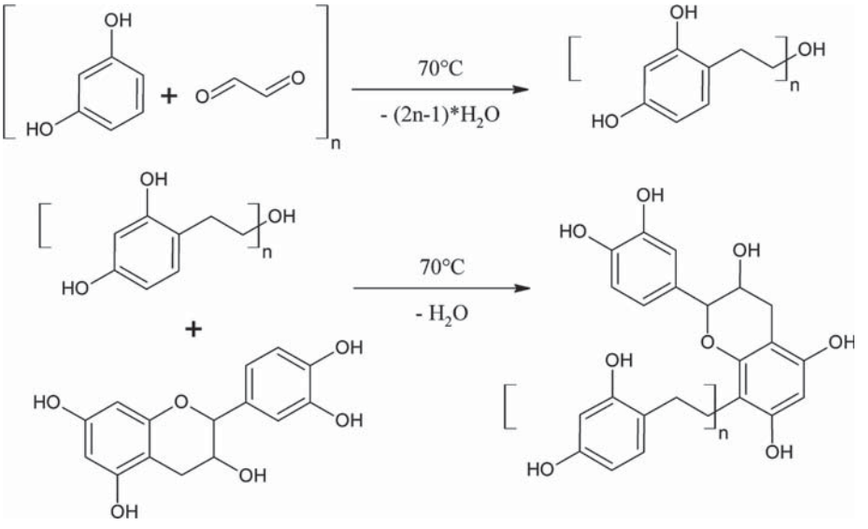


Figure 12 **A**ddition of a flavonoid on a resorcinol/glyoxal linear polymer.

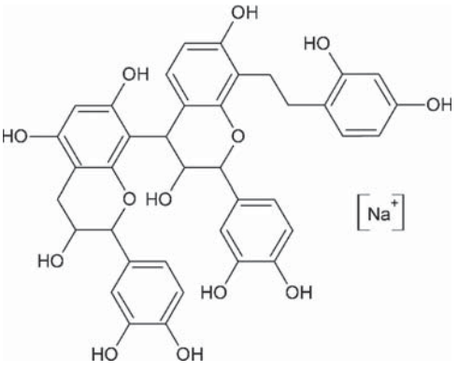


Figure 13 TRG1 polymer of 721Da (peak read at 721Da).

is possible that robinetinidins suffered the loss of an alcohol group from the pyrogallol ring in this reaction, explaining this observation [17]. The peaks for trimers are closer in intensities one to another, between 5 and 7% at a range of 1051 to 1096Da.

The fact that TRG2 molecules based on flavonoid monomers and trimers are more present than in TRG1 supports the hypothesis suggested in part 3.4 that glutaraldehyde has a better reactivity than glyoxal toward flavonoids.

Example: The peak at 763Da on Figure 3. One AB-unit oligomer, one glutaraldehyde unit and one resorcinol unit: 272 + 288 (flavonoids) + 70 (glutaraldehyde) + 108 (resorcinol) + 2*1 (-H end) + 23 (Na) = 763Da.

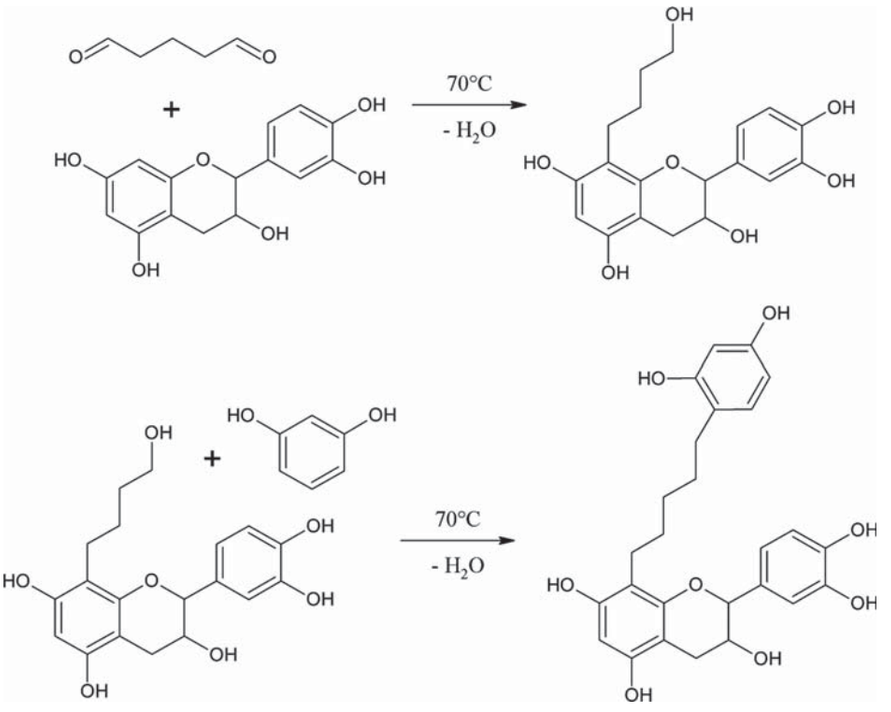


Figure 14 Tannin/glutaraldehyde/resorcinol reaction.

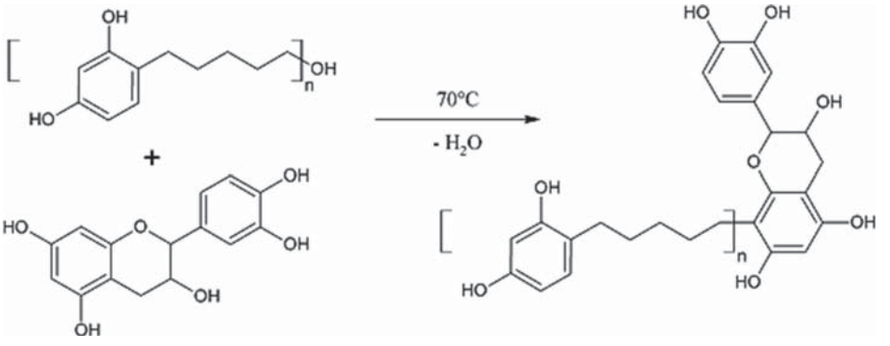


Figure 15 Addition of a flavonoid on a resorcinol/glutaraldehyde linear polymer.

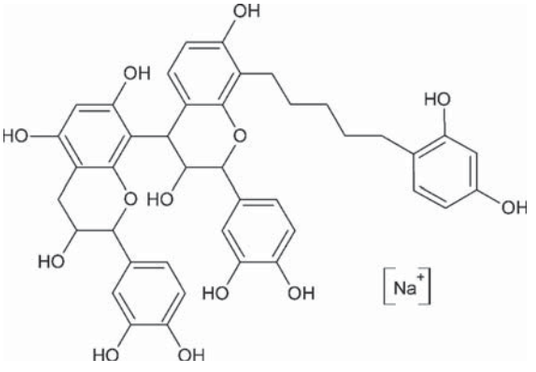


Figure 16 TRG2 polymer of 763Da (peak read at 763Da).

Both tannin-resorcinol-glyoxal and tannin-resorcinol-glutaraldehyde oligomers have molecular weight similar to the tannin-resorcinol-formaldehyde ones: from 340 to 1038Da for TRG1, 383 to 1096Da for TRG2 and 538 to 1080Da for TRF [7]. Their compositions are similar but present some differences. TRF oligomers are only made of flavonoid monomers and dimers, while trimers are visible in TRG1 and TRG2. There is always a minimum of 2 resorcinol units per TRF oligomer, up to a maximum of 4, while it is possible to see TRG1 and TRG2 oligomers having a single resorcinol unit. The TRG2 oligomers also have a maximum of 4 resorcinol units, while TRG1 oligomers have a maximum of 2 resorcinol units, possibly due do the lower reactivity of glyoxal.

4 CONCLUSION

The MALDI-ToF mass spectrometry analysis of tannin-resorcinol-glyoxal and tannin-resorcinol-glutaraldehyde resins shows that both formulations are, as in TRF resin [7], not a simple mix of resorcinol-aldehyde species and flavonoid oligomers. The tannins reacted with the other components during the curing of the resin. The use of these alternative aldehydes, however, results in some differences in the oligomers obtained at the end of the reaction. In TRF resin the resorcinol-formaldehyde oligomers are much more present than flavonoids-resorcinol-formaldehyde adducts [7] due to high reactivity of formaldehyde toward resorcinol. In TRG resins this predominance is less important, but still present: overall, the intensities of the intermediate species peaks, namely aldehydes-resorcinol, tannin-aldehydes and tannin-resorcinol, are lower than the flavonoids-resorcinol-aldehydes ones. The lower reactivity of glyoxal and glutaraldehyde is revealed by the presence of intermediate products which were not observed in TRF resin, such as flavonoid oligomers linked on their C6 or C8 to one or two aldehydes still presenting reactive sites (-CH_2OH or –CH_2^+), or resorcinol units directly bonded to the C2 of flavonoid oligomers. Eventually, this analysis shows that the lower results of these TRG adhesives compared to TRF obtained in a previous work [8] could be explained by these differences of reactivity toward phenolic compounds such as resorcinol and tannins. We can also see that glutaraldehyde has a better reactivity than glyoxal through the higher intensities of the resorcinol-glutaraldehyde and flavonoid-glutaraldehyde adducts peaks. This match has the better results of TRG2 than TRG1 that were observed [8].

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