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Supporting Information

Development of a Fast, Low-Cost, Conservative and Ecological Method for Quantifying Gallic Acid in Polymeric Formulations by FTIR Spectroscopy in Solution

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Supporting Information

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Experimental Section

Chemicals and Instruments

Gallic acid (GA), chitosan (K), polystyrene (PS), carbon tetrachloride (CCl_4) and methanol (MeOH) were purchased from Merck (formerly Sigma-Aldrich). The FTIR spectrum (KBr) of GA is herein available in Section S1, Figure S1. The FTIR spectra of the synthetically prepared GA-loaded polyester-based dendrimer (GALD) are available in Section S2 (Figure S2 and Figure S3). Data concerning GALD molecular weight (MW) and its GA loading, obtained by UV-Vis analysis and Folin-Ciocaltau method are available in Section S2, Table S1. Both reagents and solvents were used without further purifications. FTIR spectra were recorded in solution on a Perkin Elmer System 2000 spectrophotometer interfaced to a personal computer, operating under Turbo chrome workstation (version 6.1.1.). For spectra acquisition, a rectangular sealed liquid cell was adopted with NaCl rectangular windows (pathlength = 0.1 mm) and acquisitions were made in absorbance. The construction of calibration curves by least square approach was performed using Microsoft Excel (2013). UV-Vis GA determinations on GALD were performed according to the reported method[1,2] on an Agilent UV-Visible spectrophotometer Cary 100 (Varian Co., Santa Clara, CA, USA) with 0.5 nm resolution. Quartz cells of 10 mm path length were employed for containing samples solutions.

Calibration step

Construction of standard GA calibration curve

Two stock solutions of GA were prepared by dissolving GA (8.3 mg) in 11 mL of a mixture CCl_4/MeOH 10/1, thus obtaining solutions at GA concentration 0.7545 mg/mL. Dilutions 1:2, 1:3, 2:5 and 3:5 were made obtaining additional solutions at GA concentrations of 0.3773, 0.2515, 0.3018, 0.4527 and 0.4527 mg/mL respectively. The so obtained seven GA solutions were subjected to FTIR analysis. For each concentration of GA, FTIR spectra were collected by using the cell described in section 3.1. and acquisitions were made in Absorbance modality at room temperature. The resolution was set to 1 cm^{-1} and 11 scans were averaged. The wavenumber range of the spectra was from 1130 to 2000 cm^{-1}, in order to cut off some intense bands of CCl_4 and MeOH used as solvents. The area of a selected band of GA (1600-1500 cm^{-1}) was measured, by using the “peak area” tool in the software of the FTIR spectrometer. The spectra were acquired against a background spectrum acquired without both cell and sample. The spectra for each GA concentration were acquired in triplicate and the band areas was obtained as average areas ± standard deviation ($A_{average}$ ± SD) for each GA concentration. These data were used to build the standard GA calibration curve. The linear regression Equation obtained was (S1), the regression coefficient (R) was 0.9832, and the coefficient of determination (R^2) was 0.9667.

$$y = 11.258\,x - 28.976 \qquad \text{(S1)}$$

where y is the area (A) measured and x is the GA concentration (C_{GA}) (mg/mL).

Preparation of physic mixtures GA/polymer with known GA concentration

General procedure

An exactly weighted amount of GA (12.7 mg and 47.2 mg) were transferred in a mortar and an exactly weighted amount of polystyrene (81.7 mg) or chitosan (101.6 mg) were added. Then, by using the pestle of the mortar, the two solids were carefully mixed obtaining a fine dispersion of GA in the polymeric matrix. The GA contents of the mother mixtures were GA 13.45% w/w for the GA/polystyrene mixture (GAPS) and 46.50% w/w for that GA/chitosan dispersion (GAK).

Validation step: assessment of prediction ability of linear regression model by GA determination in three different GA polymer formulations

Determinations of GA content in seven samples of the two physic mixtures (GAPS and GAK) and of the synthetic GA-loaded dendrimer (GALD): general procedure

Stock solutions of GAPS, GAK and GALD were prepared by dissolving 22.1 mg (GAPS), 49.2 mg (GAK) and 8.3 mg (GALD) in 4 mL, 11 mL and 5 mL of CCl_4/MeOH, respectively 7/1, 4.5/1 and 9/1.

The solutions of GAPS, GAK and GALD were obtained at GA concentrations of 0.7425 mg/mL (GAPS), 2.08 mg/mL (GAK) and 1.23 mg/mL (GALD). Dilutions were made in order to obtain seven solutions at different GA concentrations (Table 3, Result and Discussion section in the main text and Table S2), that would fall within the concentrations range of the calibration set. The so obtained seven GAPS, GAK and GALD solutions were subjected to FTIR analysis. For each known concentration of GA in GAPS, GAK and GALD, FTIR spectra were collected in the same conditions used in the calibration phase and the area of the same band of GA (1600-1500 cm^{-1}) previously considered was measured, by using the “peak area” tool in the software of the FTIR spectrometer. The spectra for each GA concentration (C_{GA}) were acquired in triplicate and each band area was obtained as average area ± standard deviation ($A_{average}$ ± SD) for each solution analyzed. These data, reported in Table S2, were used to obtain the predicted GA concentrations (C_{GAp}) by using the Equation (S1) of the linear regression model developed.

By reporting in graphs the C_{GAp} versus the C_{GA} for each validation set separately, three linear regressions were obtained.

The linear regression Equations were (S2), (S3) and (S4) for GAPS, GAK and GALD respectively. The values of R were 0.9957, 0.9926 and 0.9968 respectively, whereas those ones of R^2 were 0.9914, 0.9853 and 0.9937 respectively. By reporting in graphs the 21 C_{GAp} of the three validation sets mixed together versus the related C_{GA}, an additional linear regression was obtained with Equation (S5), R = 0.9933 and R^2 = 0.9867.

$y = 1.0630x - 0.0245$ (S2)

$y = 0.9984x + 0.0079$ (S3)

$y = 0.9403x + 0.0429$ (S4)

$y = 0.9987x + 0.01$ (S5)

where x is the real GA concentration (C_{GAr}) (mg/mL) and y is the predicted GA concentration (C_{GAp}) (mg/mL).

Investigation step

Determinations of GA content in test samples of GAPS, GAK and GALD by using the linear regression model [Equation (S1)] and the linear regressions [Equations (S2), (S3), (S4) and (S5)]

Amounts exactly weighted of GAPS, GAK and GALD were dissolved in the same media previously used (CCl_4/MeOH 7/1, 4.5/1 and 9/1) thus obtaining solutions at C_{GA} of 0.5869 mg/mL, 0.6933 mg/mL and 0.2050 mg/mL respectively. The solutions were subjected to FTIR analysis in the conditions described in section 3.2 and the area of the same band of GA (1600-1500 cm^{-1}) was measured. The spectra for each sample were acquired in triplicate and the band areas were obtained as average areas ± standard deviation ($A_{average}$ ± SD). These data were used to obtain the C_{GAp} by using the linear regression model developed [Equation (S1)]. Then, the C_{GAp} provided by the model were used to achieve the furtherly corrected C_{GAp} by using the linear regressions constructed in the validation step [Equations (S2), (S3), (S4) and (S5)] as explained in the Results and Discussion section in the main text.

Section S1. Gallic acid (GA)

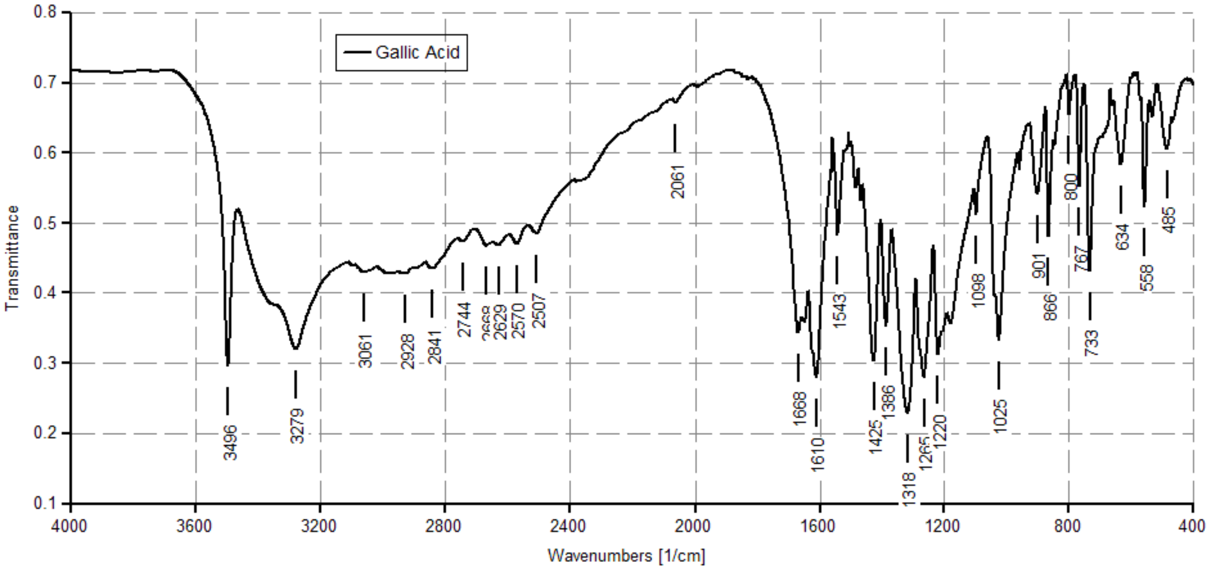

Figure S1. FTIR spectrum (KBr) of gallic acid (**1**).

Section S2. GA-loaded dendrimer formulation (GALD)

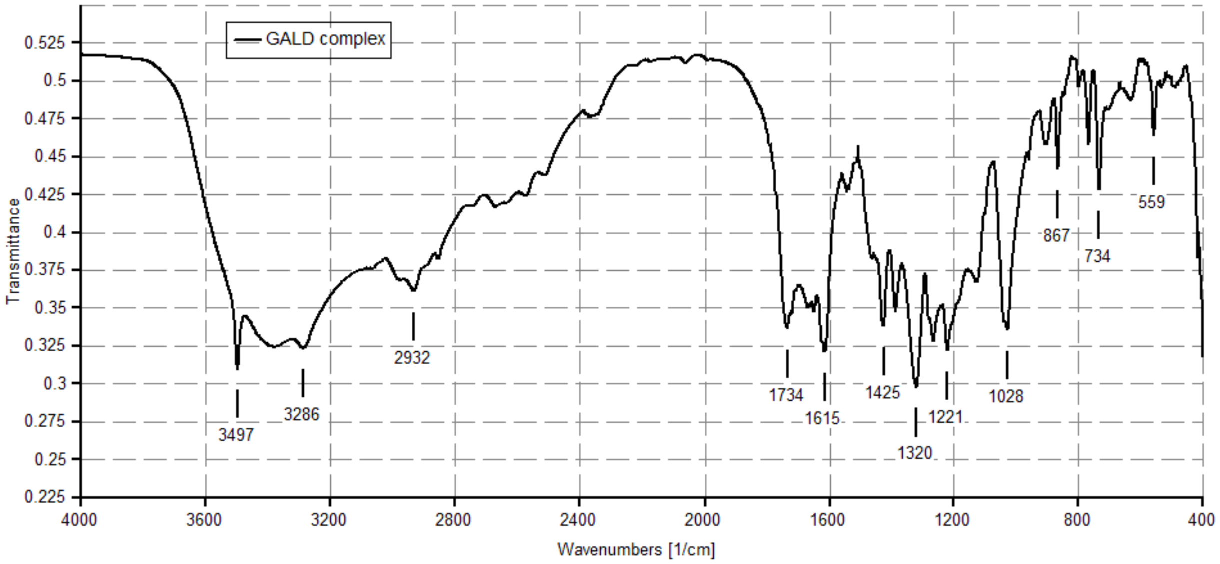

Figure S2. FT-IR spectrum (KBr) of GALD.

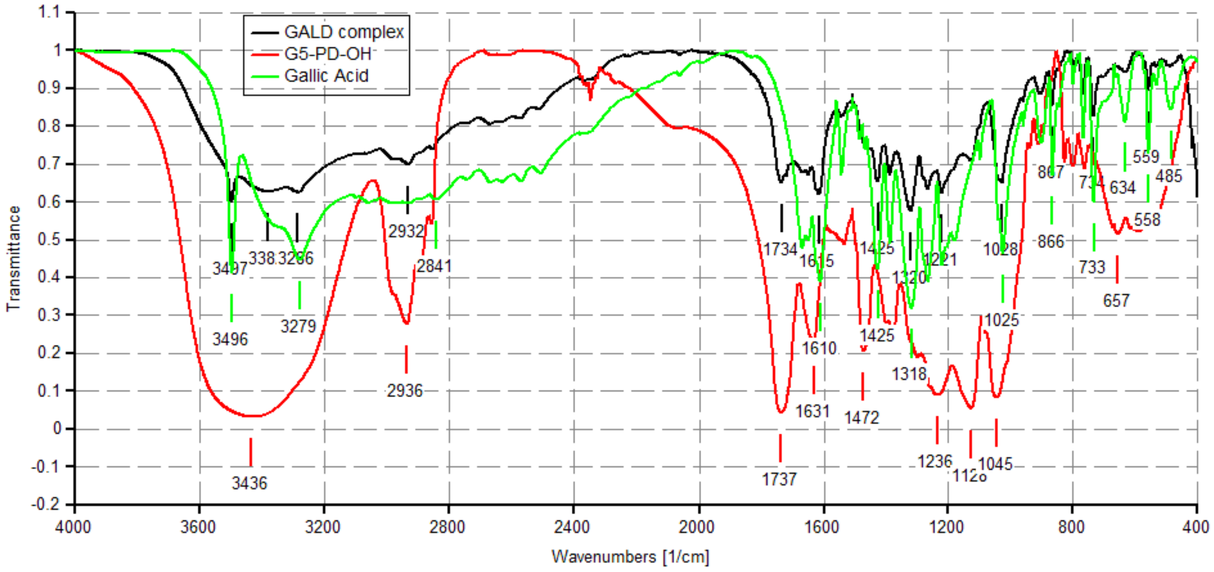

Figure S3. Comparison between FTIR spectra of GA, dendrimer scaffold (G5-PD-OH) and GALD. FTIR spectra of GA (green), dendrimer scaffold (red) and GALD complex (black) with in evidence the significant peaks.

Table S1. GA's average load ($L^{GA}_{average}$) in a sample of GALD (31.8 µg) expressed in µg, as drug loading percentage (DL%) and as GA moles (moles $_{GA}$) loaded per dendrimer mole (mole $_{D}$); computed molecular weight (MW) of GALD.

Dendrimer scaffold (µg, µmol)	$L^{GA}_{average}$ (µg, µmol)[a]	DL%	Moles $_{GA}$ mole $_{D}$	Computed MW of GALD
8.25, 0.0011	23.55, 0.138	74.1	125.5	28610

[a] By UV-Vis analytic technique and Folin-Ciocalteu method.[1,2]

Section S3. Standard GA calibration curve by FTIR spectroscopy

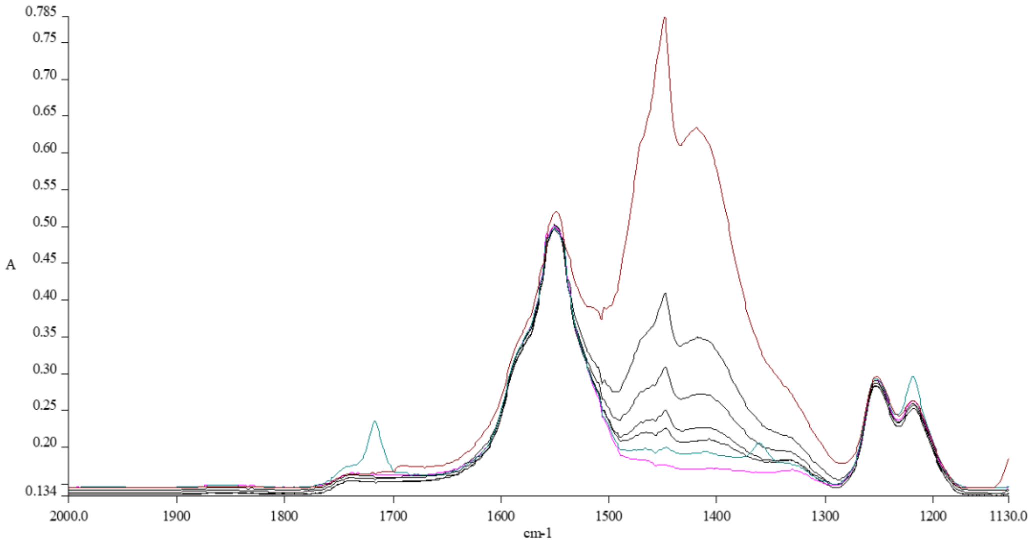

Figure S4. FTIR spectra of GA dissolved in CCl_4/MeOH at different GA concentrations used to build up the standard GA calibration curve.

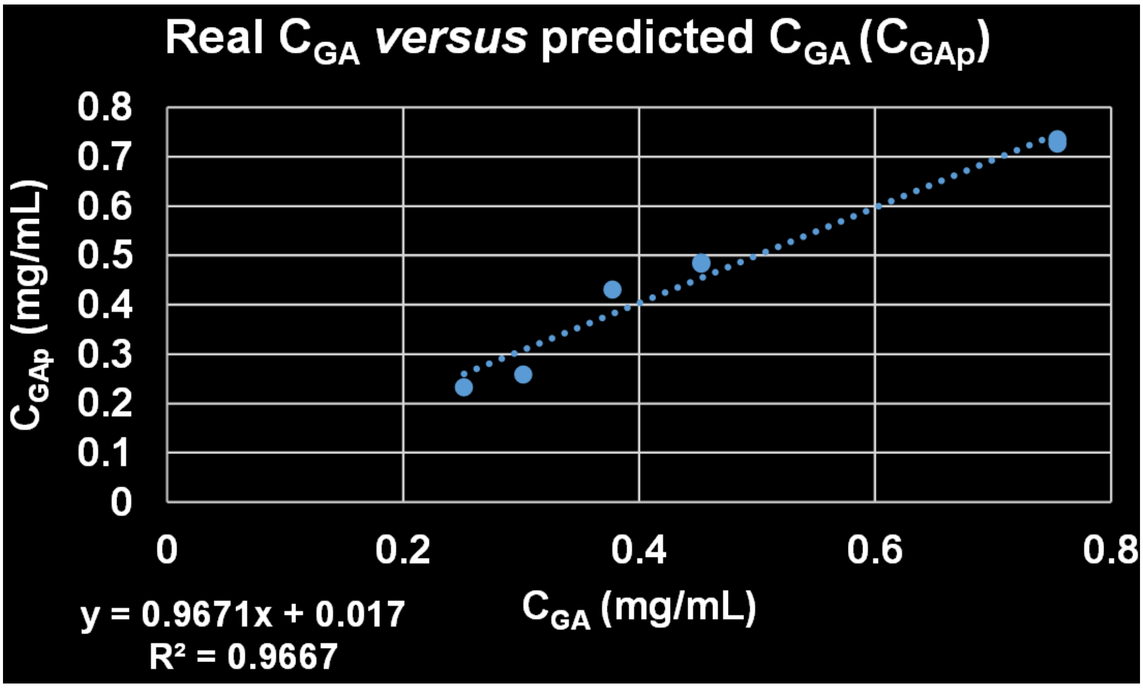

Figure S5. Real GA concentrations (C_{GA}) versus predicted ones (C_{GAp}).

Section S4. Validation of GA calibration curve on three GA polymer formulations

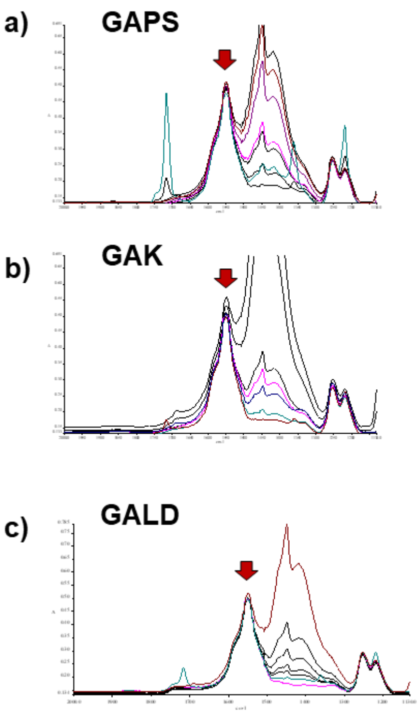

Figure S6. FTIR spectra of GAPS (a), GAK (b) and GALD (c) in solution (CCl_4/MeOH) at different GA concentrations.

Table S2. Validation step significant data.

$C_{GA/GAPS}$ (mg/mL)	$A_{average}$	$C_{GAp/GAPS}$ (mg/mL)	Residuals[a]	$C_{GA/GAK}$ (mg/mL)	$A_{average}$	$C_{GAp/GAK}$ (mg/mL)	Residuals[a]	$C_{GA/GALD}$ (mg/mL)	$A_{average}$	$C_{GAp/GALD}$ (mg/mL)	Residuals[a]
0.7425	37.5106	0.7581	0.0156	0.2600	32.1260	0.2798	0.0198	0.2589	32.2194	0.2881	0.0292
0.5940	35.8186	0.6078	0.0138	0.2971	32.0798	0.2757	0.0214	0.2733	32.3714	0.3016	0.0283
0.4950	34.8459	0.5214	0.0264	0.3467	33.1572	0.3714	0.0247	0.3075	32.6349	0.3250	0.0175
0.4243	33.5445	0.4058	0.0185	0.4160	33.7730	0.4261	0.0101	0.4100	33.9464	0.4415	0.0315
0.3713	33.3835	0.3915	0.0202	0.5200	34.6568	0.5046	0.0154	0.4920	34.6410	0.5032	0.0112
0.2970	32.0483	0.2729	0.0176	0.6933	36.9557	0.7088	0.0155	0.6150	35.7094	0.5981	0.0169
0.2700	31.9763	0.2665	0.0035	0.3782	33.4285	0.3955	0.0173	0.7029	37.0806	0.7199	0.0170

[a] Absolute value.

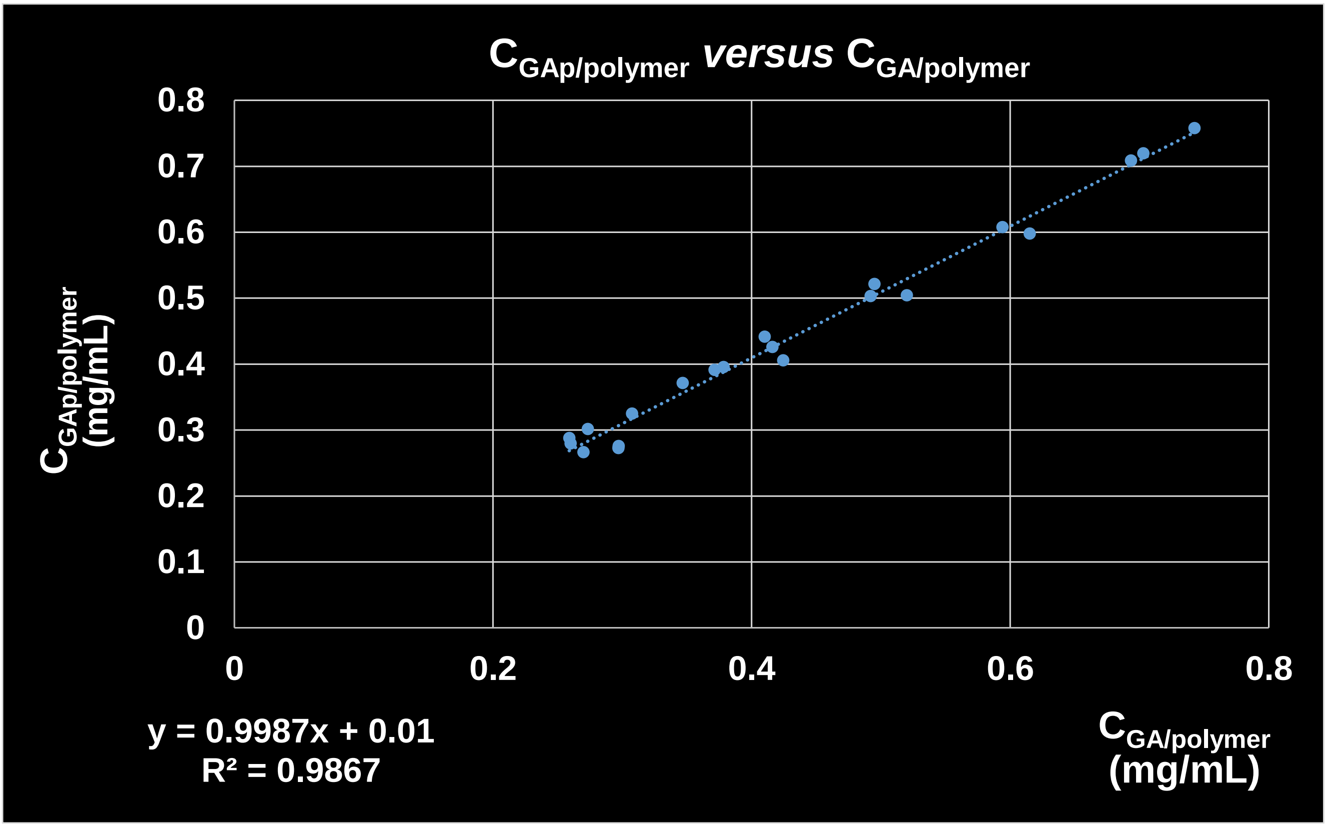

Figure S7. $C_{GAp/polymer}$ versus $C_{GA/polymer}$

Table S3. Corrected data of GA determinations in samples of GAPS, GAK and GALD at known concentrations by using Equations of linear regressions constructed in the step of validation.

Equations		**2**	**3**	**4**	**5**		
Sample	C_{GA} (mg/mL) (% w/w)	$C_{Gap/GAPS}$ (mg/mL) (% w/w)	$C_{Gap/GAK}$ (mg/mL) (% w/w)	C_{GAp} (mg/mL) (% w/w)	C_{GAp} (mg/mL) (% w/w)	Absolute error (mg/mL)	Residuals (% w/w)
GAPS	0.5869	0.6014			0.6056	0.0145 (2)[a]	0.34 (2) [a]
	13.45	13.79			13.89	0.0187 (5) [a]	0.44 (5) [a]
GAK	0.6933		0.6975		0.6952	0.0042 (3) [a]	0.31 (3) [a]
	46.50		46.81		46.66	0.0019 (5) [a]	0.16 (5) [a]
GALD	0.2550			0.2438	0.2624	0.0112 (4) [a]	3.30 (4) [a]
	74.1[b]			70.8	76.2	0.0074 (5) [a]	2.10 (5) [a]

[a] Equation used. [b] Obtained by UV-Vis determination.

References

[1] V. L. Singleton, J. A. Rossi, *Am. J. Enol. Vitic,* **1965**, *16*, 144-158.
[2] F. Turrini, R. Boggia, D. Donno, B. Parodi, G. Beccaro, S. Baldassari, M. G. Signorello, S. Catena, S. Alfei, P. Zunin, *Eur. Food Res. Technol.,* **2020**, *246*, 273-285.