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Selective control of the radical-scavenging activity of poly(phenols) in aqueous media in terms of their electron-donor properties, using a stable organic radical as chemical sensor

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ABSTRACT

The tri-potassium salt of tris(2,3,5,6-tetrachloro-4-hydroxysulphonylphenyl)methyl radical, $3K^+$ $TSPTM^{3-}$, is a good chemical sensor of the radical-scavenging activity of poly(phenols) in aqueous media. Its water solubility and its stability at all pH values facilitates the study of the influence of the pH of the medium on the activity of poly(phenols). The radical-scavenging activity of three flavonoids of the catechol and pyrogallol type and the activity of catechol, pyrogallol and methyl gallate at pH values 7 and 8 which are close to physiological pH value (7.4) have been measured. This radical species reacts exclusively by an electron transfer mechanism against the reducing poly(phenols). Therefore, the experiments to determine the radical-scavenging activity of poly(phenols) with $3K^+$ $TSPTM^{3-}$ are related to their ionization potentials (IP). As IP is a function of the pH of the medium, the electron donor ability of a poly(phenol) depends on the acidity or basicity of the fluid in question. The radical-scavenging activity of poly(phenols) **1–6** is higher at pH 8 than at pH 7. $3K^+$ $TSPTM^{3-}$ is able to discriminate between catecholic and pyrogallolic moieties of poly(phenols) in water solutions by taking measurements at different pH values. By adjusting the pH of the medium $3K^+$ $TSPTM^{3-}$ will easily detect those moieties (e.g. pyrogallol) most prone to elicit prooxidant effects.

1. Introduction

Poly(phenols) (ArOH) are defined as those species with more than one hydroxyl group in one or more phenol moieties per molecule. Many natural poly(phenols) are free radical scavengers with application as food antioxidants and possible antioxidant effect in vivo. The radical-scavenging activity seems to be related to the molecular structure and more precisely to the number and position of hydroxyl groups. They are capable of scavenging reactive oxygen species (ROS) such as LOO•, HO•, O_2•$^-$ and NO• inter alia, through hydrogen atom donation with formation of a more stable phenoxyl radical, or through a mechanism consisting of a single-electron transfer from ArOH or its phenoxide anion (ArO^-) to ROS [1]. The first process is related to the hydrogen atom-donating ability of the antioxidant and the stability of the radical species ArO• formed, which are characterized by the bond-dissociation energy of the phenolic O–H bond. The second process corresponds to the electron-donating ability of the antioxidant in its molecular (ArOH) or anionic (ArO^-) form and closely related to the ionization potential of the neutral or anionic molecule. However, poly(phenols) can also exert prooxidant effects, depending on several different factors, inter alia the nature and concentration of poly(phenols), the solvent and the pH of the medium. The oxidative activity of plant poly(phenols) is primarily associated with the generation of superoxide radical anion (O_2•$^-$) radical anion in the presence of oxygen or with the ability to reduce metallic ions such as Fe(III) or Cu(II) in the biological systems. The O_2•$^-$ ultimately leads to hydrogen peroxide H_2O_2 formation in the presence of superoxide dismutase, and the reduced Fe(II) or Cu(I) can be oxidized back with H_2O_2 in the Fenton-type reaction leading to the production of HO• as one of the most harmful ROS (Scheme 1).

Generally, poly(phenols) with strong radical-scavenging activity are oxidized at relatively low potentials [2]. As a low oxidation potential is also associated with a strong electron-donating ability, the balance between antioxidant:prooxidant activity of a poly(phenol) is difficult to predict and depends on the particular conditions of each case. For instance, some flavan-3-ols such as (−)-epicatechin act as antioxidants at low concentrations but have an oxidant effect at high concentrations [3]. As poly(phenols) have several dissociable OH groups in the molecular structure, it is expected that the pH of the medium will influence their radical-scavenging activity. Thus, the observed reaction rates of flavan-3-ols to inactivate the superoxide radical

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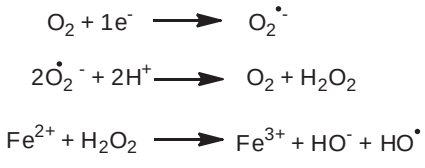


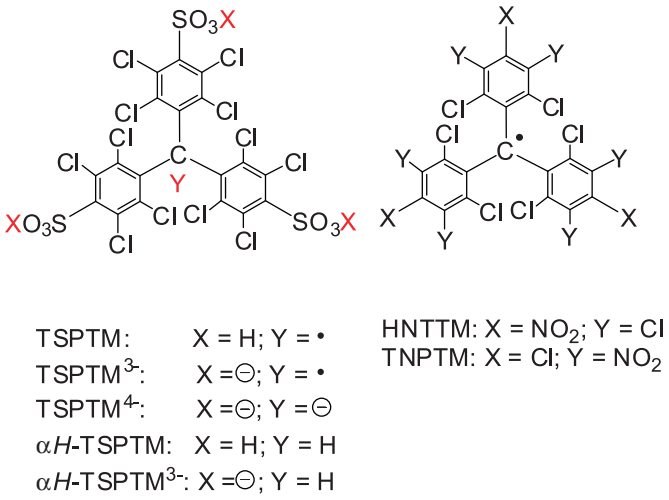
Scheme 1.

anion are different in the pH range and vary from pH 7 to pH 10 [4] and, in general, are deeply affected by the pH of the medium [5]. The radical-scavenging activity of the catechins increases with increasing pH of the medium [6]. This pH dependence of the activity of flavan-3-ols is closely related to the acid dissociation of the different hydroxyl (OH) functions of the poly(phenolic) structure, since the deprotonated phenolic (ArO^-) group have stronger electron-donating properties than the protonated (ArOH) one [2,7,8].

The antiradical character of the basic and more simple polyhydroxybenzenes, catechol and pyrogallol, as the most active moieties in the molecular structure of most natural poly(phenols), can predict at least in part the antioxidant/prooxidant activities of the more complex poly(phenols). Both of them show a characteristic and different electrochemical behaviour. The cyclic voltammetry of catechol shows a single and quasi-reversible two-electron two-proton process, the anodic peak being related to the oxidation to *o*-quinone [9]. Some authors claimed that this quasi-reversible electrochemical process corresponds to an oxidation reaction involving only one electron. In such a case, the intermediate aryloxyl radical as one-electron oxidation product of catechol undergoes disproportionation leading to the formation of *o*-quinone and the regeneration of the starting catechol [10]. On the other hand, the cyclic voltammetry of pyrogallol displays an irreversible oxidation peak, and the absence of the cathodic peak in the reverse scan indicates an EC mechanism, i.e. an oxidation process in the anode followed by a chemical reaction which rapidly removes the product generated on the anode.

The oxidation potential (E_p^a) value of a poly(phenol) in the cyclic voltammetry is closely related to its radical-scavenging activity as antioxidant [2]. The lower the value of E_p^a, the higher the radical-scavenging activity. On the other hand, E_p^a is also correlated with the prooxidant activity of the poly(phenols). The lower the value of E_p^a, the easier the electron-donating ability. In general, the E_p^a values of poly(phenols) in aqueous solution decrease with the increase of pH and are closely related to the acid dissociation constants (pK_a) of them. In general, poly(phenols) with small pK_a show high antioxidant activities [2,5,8].

In the initial chemical stage of an electron transfer process, the oxidation of the poly(phenol) leads to the formation of phenoxyl radical. These new species may in turn be suitable for further oxidations, transferring again an electron to the chemical sensor, or undergoing chemical transformations to new species with oxidative potentials low enough to continue the electron transfer processes. Consequently, these reactions result in most cases very complex and give rise to multiple stoichiometries. Therefore, it is necessary to distinguish different stoichiometric values. The *total stoichiometry* (TS) of the process is determined from the overall amplitude of the reaction. Moreover, as the reaction rate of these radical-scavenging processes against the very short-lived free radicals in the living organism is a key factor to qualify a poly(phenol) as an antioxidant, the *kinetic stoichiometry* (KS) of the process is defined as the number of electrons transferred during the first fast part of the reaction [11]. A good example of a complex radical-scavenging process in acidic aqueous media is the reaction of the tri-anion of the tris(tetrachloro-4-sulfophenyl)methyl radical ($TSPTM^{3-}$) (Scheme 2) with a simple



Scheme 2.

poly(phenol) such as pyrogallol. The total stoichiometry of this reaction is found to be around 6–7 mols of $TSPTM^{3-}$ radical per mol of pyrogallol [12].

We have been engaged for a time with the synthesis of stable organic free radicals as chemical sensors of the antioxidant power of poly(phenols). In this context, HNTTM and TNPTM (Scheme 2) have shown to be sensitive to the electron transfer reactions from poly(phenols) [13,14,15]. The oxidative capacity of these radicals is intimately related to their electrochemical cathodic peak potential (E_p^c). Thus, HNTTM with a high potential (E_p^c=0.50 V) oxidizes catechol, pyrogallol and those poly(phenols) with catechol or pyrogallolic moieties. In turn, TNPTM with a lower potential (E_p^c=0.14 V) is able to discriminate between pyrogallol and catechol, being only active with pyrogallol. More recently, we have reported the synthesis of a new stable and water-soluble radical of the PTM (perchlorotriphenylmethyl) series, TSPTM, and its radical-scavenging capacity in front of catechol, pyrogallol and ascorbic acid, in acidic aqueous solutions [12]. UV–vis absorption spectrum of TSPTM has two bands associated with the radical character with maxima at $\lambda(\varepsilon)$, 385(17 280) and 503(740) nm, and shows a single and broad band in the epr spectrometer at $g=2.0032\pm0.0005$. It is a very stable radical in hydrogen atom transfer reactions, unlike most of the free radicals, but is very sensitive to electron donors in electron transfer processes. Its difficulty to abstract hydrogen atoms from hydrogen-donating reagents is accounted for by the steric hindrance of the bulky polychlorophenyl substituents around the trivalent carbon atom [16]. Upon reduction by electron transfer, the charged species $TSPTM^{4-}$ (Scheme 2) is rapidly protonated to $TSPTM^{3-}$. The tetraanion $TSPTM^{4-}$ is stable in basic solutions, showing a UV–vis characteristic absorption maximum at λ=535 nm. These reductive processes from the radical to the anion can be conveniently monitored by UV–vis and electron paramagnetic resonance (epr) spectra. A great advantage in the use of TSPTM as a chemosensor of the antiradical activity of poly(phenols) with regard to the use of diphenylpicrylhydrazil radical (DPPH) in methanol, a standard method widely used in the literature, is that the experiments with TSPTM can be carried out in aqueous media that more closely resemble the environment in biological fluids. The high solubility in water is due to the presence of three sulphonic functions, $-SO_3H$, on the phenyl rings which are completely dissociated in water. Some reported preliminary results show that ascorbic acid presents a radical-scavenging activity with $TSPTM^{3-}$ in acidic (pH$\sim$3) aqueous solution [17], (rate constant of the second order reaction, $k=33\ M^{-1}\ s^{-1}$),

pyrogallol exhibits a significantly low activity ($k=1.5\ M^{-1}\ s^{-1}$) and the activity of catechol is noticeably lower ($k=0.05\ M^{-1}\ s^{-1}$) [12]. These results demonstrate the potential use of the $TSPTM^{3-}$ as a chemical sensor of antioxidants, and the advantage of assays using this radical is that it can discriminate between pyrogallol and catechol derivatives. As the reactions proceed by electron transfer from the antioxidant to $TSPTM^{3-}$, the order of reactivity ascorbic acid > pyrogallol > catechol is well correlated with their anodic peak potentials. As the reactions proceed in acidic aqueous media (pH∼3), catechol ($pK_a=9.4$, for OH deprotonation) [18] and pyrogallol ($pK_a=8.9$) [18] are practically in their neutral form, and the electron donation becomes much more difficult. Only ascorbic acid ($pK_a=4.0$) [19] is partially deprotonated at pH=3, and the electron transfer from the ascorbate anion is much easier than from the neutral form. The aqueous solution experiments at different pH will allow to study the influence of the acidity or basicity of the medium in the kinetics of the antiradical processes. It is expected that the raise of the pH of the aqueous solutions will increase the radical-scavenging activity of the (poly)phenols. It is worth mentioning that the rate constant for the tocopherol-regeneration reaction by ascorbic acid, a powerful antioxidant, increased with increasing pH value [20].

Consequently, we now report the synthesis and characterization of TSPTM tripotassium salt, $3K^+$ $TSPTM^-$, a salt of neutral hydrolysis, to investigate the antioxidant activity of (−)-epicatechin and (−)-epigallocatechin, two flavanols containing catechol and pyrogallol moieties, respectively, at two pH values (7 and 8), which are close to physiological pH (7.4) and may therefore be valuable in determining relative antioxidant activity in biological systems. These pH values are favourable to deprotonate the most dissociable OH groups of the molecules. Their activities are compared at the same pH values with those of catechol and pyrogallol as models of the above flavanols, with quercetin as a flavonol widely distributed in the nature, and with methyl gallate.

2. Experimental

2.1. General

IR spectra were recorded with a FT-IR spectrophotometer, electronic spectra with a single cell UV–visible spectrophotometer and the EPR spectra with a EMX-Plus 10/12 spectrometer. Electrospray mass spectra (ESI-MS) were recorded on a LC/MSD-TOF and m/z referenced to 35Cl. The potassium content was determined by inductively coupled plasma (ICP) in a multichanel instrument under standard conditions.

2.2. Synthesis of the tri-potassium salt of tris(2,3,5,6-tetrachloro-4-hydroxysulphonylphenyl)methyl radical ($3K^+$ $TSPTM^{3-}$)

To a stirred solution of α*H*-TSPTM (508 mg; 0.64 mmols) in THF-H_2O (5:1) (60 mL) at room temperature, an aqueous solution of KOH (1 M) (2.5 mL) was added and the strong red solution was stirred overnight. Chloranil (0.528 g; 2.1 mmol) was added in one portion and the solution was stirred further (6 h). Afterwards, the solution was evaporated at reduced pressure and the residue was digested twice in ethyl acetate–methanol (2:1). The insoluble fraction, separated by filtration and dried, gave $3K^+$ $TSPTM^{3-}$ (592 mg, 98%) as a red solid: IR (KBr, cm^{-1}) 1307 (s), 1223 (s), 1117 (m), 1067 (s), 614 (s); UV–vis (H_2O) λ_{max}/nm (/$Lmol^{-1}\ cm^{-1}$) 368 (sh) (8970), 385 (17 500), 504 (745), 556(sh) (615). Anal. Calcd. for $C_{19}Cl_{12}O_9S_3K_3$ (1011.029) C, 22.57; K, 11.60. Calcd. for $C_{19}Cl_{12}O_9S_3K_3\cdot KOH$ (1067.18) C, 21.4; K, 14.6. Found: C, 22.03; K, 15.45. ESIHRMS (−) calcd. for $[C_{19}HCl_{12}O_9S_3]^{2-}$ $(M+H)^{2-}$ 444.2541, found 444.2528; calcd for $[C_{19}HCl_{12}O_6S_2]^{2-}$ $(M+H-SO_3)^{2-}$ 404.2743, found 404.2731; calcd for $[C_{19}Cl_{12}O_9S_3]^{-3}$ $(M)^{3-}$ 295.8328, found 295.8302.

2.3. Preparation of buffer solutions

Solution 1 (Na_2HPO_4 25 mM and NaCl 0.2 M): 3.55 g of Na_2HPO_4 and 11.69 g of NaCl were taken up to 1 L with water. *Solution 2* (citric acid 25 mM and NaCl 0.2 M): 5.25 g of Citric acid and 11.69 g of NaCl were taken up to 1 L with water. **Buffer solutions were at pH 8.02 and 7.00**. To solution 1 (250 mL) solution 2 was added to achieve the desired pH.

2.4. Kinetic measurements

All the experiments were monitored by UV–vis spectroscopy at room temperature and the buffer solutions at pH 7.00 and 8.02 were freshly prepared and completely deoxygenated with Ar. Equal volumes (1 mL) of a solution of K_3TSPTM (final concentration, 140 μM) and poly(phenol) (final concentration, 14 μM) were placed in the spectrometer cell. The decay of the band intensity ($\lambda=385$ nm) was continuously recorded. The rate constants and the number of electrons transferred in the first and/or second processes and over a period of 2 h were estimated with the general kinetic model of second order reported by Goupy et al. defined by Eq. (1) [21]. The values for the rate constant, k, were calculated from the integrated Eq. (2).

$$-d[radical]/dt = k\cdot n\cdot[polyphenol][radical] \quad (1)$$

$$ln\frac{1-A_f/A}{1-A_f/A_0} = -\frac{knc}{A_0/A_f-1}t \quad (2)$$

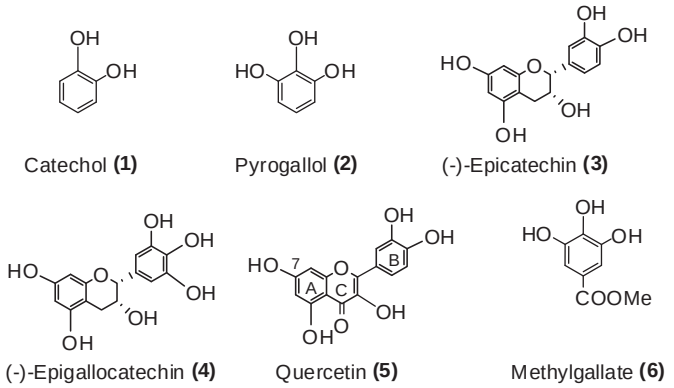
In Eqs. (1) and (2), n represents the number of reduced moles of radical per mole of poly(phenol); A_o is the initial intensity of the radical signal in the UV–vis spectra; A_f is the final visible intensity; and c is the initial concentration of poly(phenol).

All the graphs were manipulated using OriginPro 7 software. One or two processes, depending on the poly(phenol), were defined through a complete analysis of the overall reaction. Values for the first fast step of the reaction (k_1, n_1), for the second and slower process (k_2, n_2) and for the total stoichiometry over a period of 2 h were calculated to estimate the antioxidant power of the poly(phenol).

3. Results and discussion

The α*H*-TSPTM (Scheme 2) was prepared as previously described [12]. The tri-potassium salt of $TSPTM^{3-}$, $3K^+$ $TSPTM^{3-}$, was prepared, isolated and characterized as described in the Experimental part. The water solutions of this salt are neutral unlike the acid solutions of TSPTM (160 μM, pH∼3.4) [12].

The radical-scavenging acivity of simple poly(phenols) such as catechol (**1**), pyrogallol (**2**) and methyl gallate (**6**), flavanols such as (−)-epicatechin (**3**) and (−)-epigallocatechin (**4**), and the flavonol quercetin (**5**) (Scheme 3) was measured against $3K^+$ $TSPTM^{3-}$ as the chemical sensor in buffer solutions at pH 7 and 8. The reactions were monitored by the decrease of the intensity of the absorption band in the UV–vis spectrum ($\lambda=385$ nm) of $TSPTM^{3-}$. The number of electrons transferred per molecule of antioxidant was higher than 1 for all the reactions. The experiments were performed in an excess of $3K^+$ $TSPTM^{3-}$ ($3K^+$ $TSPTM^{3-}$:antioxidant molar ratio, 10:1) to calculate the total stoichiometric factor of the reactions. There are two or more stages in most of these reactions, the former being faster than the latter, and the decay in the absorbance of the $TSPTM^{3-}$ continued



Scheme 3.

Table 1
Observed rate constants (in $M^{-1}\,s^{-1}$) and stoichiometric factors for the reaction of the tri-potassium salt of $TSTTM^{3-}$, $3K^+$ $TSPTM^{3-}$, with catechol, pyrogallol, (−)-epicatechin, (−)-epigallocatechin, quercetin and methyl gallate at a molar proportion of ~10:1, in aqueous buffer solutions at two different pH values[a,b].

Poly(phenol)	*pH*	1st process		2nd process		*TS*[e]/2 (h)
		k_1	KS_1[c]/*t* (min)[d]	k_2	KS_2[c]/*t* (min)[d]	
Catechol	7	0.56	0.87/75	0.20	0.79/420	0.94
	8	3.24	0.65/29	0.50	2.46/620	1.10
Pyrogallol[f]	7	13.72	4.8/60			7.67
	8	7736.3	0.72/0.5	147.6	0.90/45.5	5.3
(−)-Epicatechin[g]	7	0.45	2.7/417			0.87
	8	0.94	3.67/260			2.10
(−)-Epigallocatechin[f]	7	6.72	4.44/60			5.27
	8	3137.9	0.94/1	1.20	2.10/13.6	3.65
Quercetin[h]	7	–	–	–	–	–
	8	389.47	2.57/24	7.27	1.07/35	3.89
Methyl gallate[f]	7	3.83	1.54/60			2.67
	8	1523.5	0.84/1	3.96	1.81/37	4.3

[a] Initial concentrations of the reactants: $3K^+$ $TSPTM^{3-}$, 140 μM; poly(phenol), 14 μM.
[b] Data are the averages of at least three determinations with a deviation of less than ± 12%.
[c] Kinetic stoichiometric factors for this particular process.
[d] Reaction time of the process.
[e] Total stoichiometric factor after 2 h of reaction.
[f] A single and simple process is identified in the reaction at pH 7.
[g] Single and simple processes are identified in the reaction of (−)-epicatechin at pH 7 and 8.
[h] Quercetin is not soluble in buffer solution at pH 7.

slowly over a long period of time, sometimes consuming nearly all the radical in the medium. This high value on the stoichiometry of the reactions may be due to the activity of secondary products, formed by dimerization and/or polymerization of the first radical species ArO• generated from the starting material, or formed by reaction of quinones—generated by oxidation of the starting material—with the nucleophilic solvent. These secondary products with sufficiently low oxidation potential values should be capable of further reacting with the radical by electron transfer. The radical-scavenging activity of a poly(phenol) is related to both the stoichiometry of the reaction, i.e. the number of electrons that are transferred from the poly(phenol), and the reaction rate. Table 1 shows the values of the rate constants and kinetic stoichiometric factors for the first and secondary processes of the reactions, and the total stoichiometric (TS) factors over a period of 2 h.

All the poly(phenols) studied possess various dissociable OH groups in their structure and it was expected an equilibrium between the neutral and the deprotonated form of the most acidic

Table 2
Literature pK_a values for the first OH group of poly(phenols) and molar fractions of the base-acid species in equilibrium at pH 7 and 8.

Poly(phenol)	**1**	**2**	**3**	**4**	**5**	**6**
pK_a	9.40[a]	8.94[a]	8.72[b]	7.73[c]	7.70[d]	8.03[e]
$\frac{[ArO^-]}{[ArOH]}$ pH = 7	0.004	0.01	0.02	0.2	0.2	0.09
$\frac{[ArO^-]}{[ArOH]}$ pH = 8	0.04	0.1	0.2	1.9	2.0	0.9

References:
[a] [18].
[b] [2,22].
[c] [8,23].
[d] [24].
[e] [2,25].

OH group in buffer solutions at pH 7 and 8.

$$[ArOH] \rightleftharpoons [ArO^-] + [H^+]$$

This equilibrium is defined by the acid dissociation constant (pK_a) value, and the relation between the pK_a and the pH of the medium is given by the Henderson Hasselbalch equation:

$$pH = pK_a + \log\frac{[ArO^-]}{[ArOH]}$$

$$\textit{if}: \quad pH = pK_a, \ \text{then}: \quad [ArO^-] = [ArOH]$$

A list of the pK_a of the poly(phenols) and of the molar fractions of the base-acid species in equilibrium at pH 7 and 8 is displayed in Table 2. As the pK_a values are in the proximity of the pH of the buffer solutions, the poly(phenols) must occur as a mixture of molecular [ArOH] and ionized [ArO^-] species with similar concentrations. The equilibrium between both species is shifted to the left, $[ArOH] \gg [ArO^-]$, in poly(phenols) **1–3** at pH 7 and to the right, $[ArO^-] > [ArOH]$ at pH 8 in poly(phenols) **4–6**. As the electron-donor capacity of a compound is closely related to the ionization potential (IP), and this value is higher for the deprotonated than for the molecular form, the radical-scavenging activity of these poly(phenols), referred to k_1 values of the first process of the reaction, is strongly dependent on the pH of the medium and, higher at pH 8 than at pH 7, as shown in Table 1.

An important factor governing the radical-scavenging activity of the poly(phenols) is the number and position of hydroxyl groups. The pyrogallol moiety in (−)-epigallocatechin and methyl gallate confers more scavenging activity than the catechol moiety in (−)-epicatechin. In agreement with this, catechol and (−)-epicatechin show a very low activity against $3K^+$ $TSPTM^{3-}$ whereas methyl gallate, pyrogallol and (−)-epigallocatechin readily react (very high rate constant values in the first stage) at pH 8. Moreover, pyrogallol and the pyrogallolic derivatives **4** and **6** present high total stoichiometric values after 2 h of reaction. This confirms the complexity of these processes. Quercetin with a catechol moiety in the molecular structure shows more activity in buffer solution at pH 8 than catechol (**1**) and (−)-epicatechin (**3**) because one of the HO groups from the catechol moiety (ring B) and the 7-OH group on the other benzene ring (A) (Scheme 2), which seems the most acidic OH group of the molecule [26], are *para*-conjugated with an α-hydroxy-α,β-enone and carbonyl moieties, respectively.

Graphics corresponding to the reaction of pyrogallol (**2**) and (−)-epigallocatechin (**4**) with $3K^+$ $TSPTM^{3-}$ are illustrated in Figs. 1 and 2, respectively, where the time evolution of the intensity of the band in the UV–vis spectroscopy of the $TSPTM^{3-}$ and an estimation of the second order kinetics are shown. Kinetic graphics for methyl gallate (**6**), catechol (**1**) and (−)-epicatechin (**3**) are displayed in the Supporting Information. An analysis of the graphics for **2**, **4**, and **6** at pH 8 show a sharp drop of intensity in

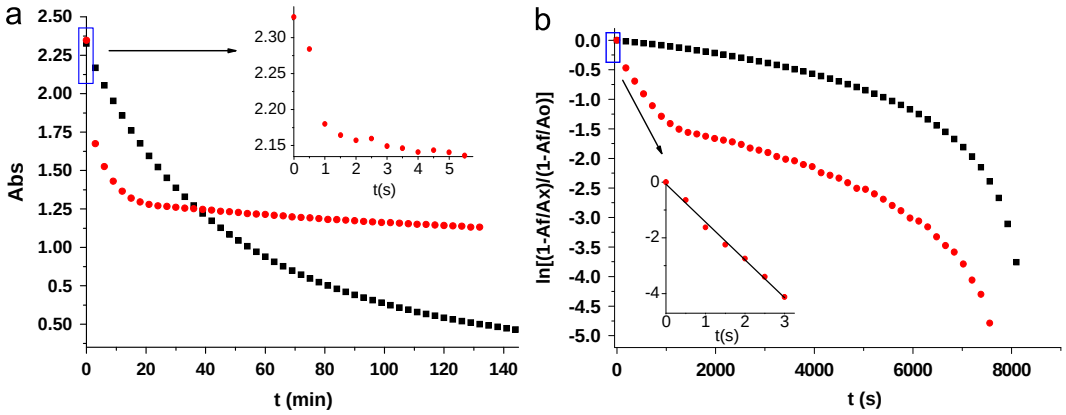


Fig. 1. (a) Decay of the intensity of the band in the UV–vis spectrum of a buffer solution (black at pH=7, red at pH=8) of $3K^+$ $TSPTM^{3-}$/pyrogallol (molar ratio, 10:1); (inset): a detailed decay of the intensity at very short times at pH=8. (b) Estimation of the second order kinetics at pH=7 (black) and at pH=8 (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

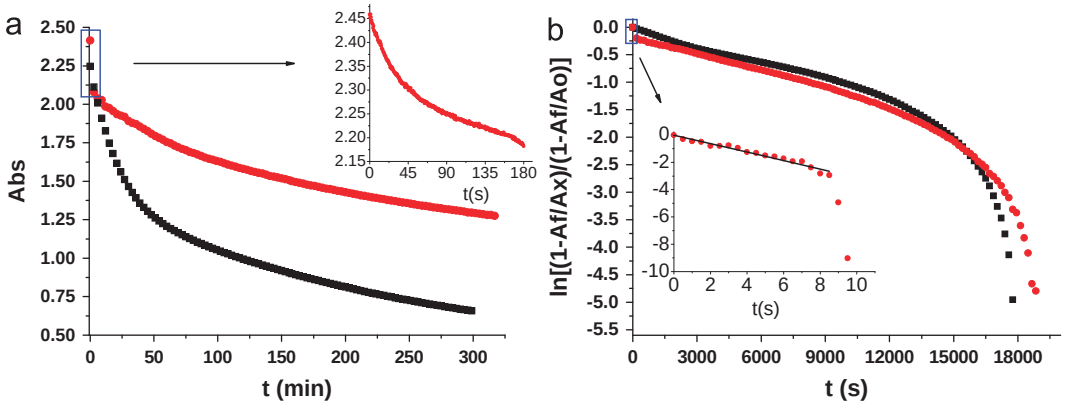


Fig. 2. (a) Decay of the intensity of the band in the UV–vis spectrum of a buffer solution (black at pH=7, red at pH=8) of $3K^+$ $TSPTM^{3-}$/(−)-epigallocatechin (molar ratio, 10:1); (inset): a detailed decay of the intensity at very short times at pH=8. (b) Estimation of the second order kinetics at pH=7 (black) and at pH=8 (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

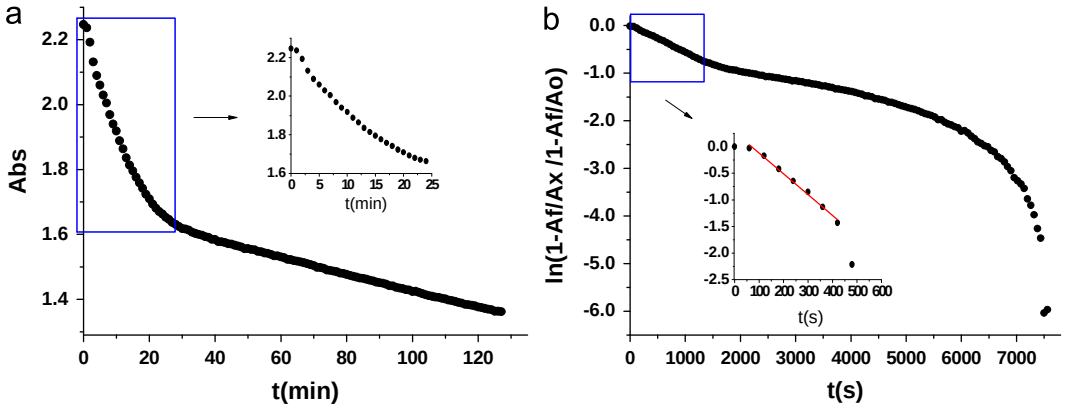


Fig. 3. (a) Decay of the intensity of the band in the UV–vis spectrum of a buffer solution at pH=8 of $3K^+$ $TSPTM^{3-}$/(−)quercetin (molar ratio, 10:1); (inset): a detailed decay of the intensity at very short times. (b) Estimation of the second order kinetics at pH=8.

the very first stage which results in a high value of the rate constant k_1, followed by a second less pronounced stage with a moderate value of the rate constant k_2. Finally, the reaction goes further to complete the number of electrons transferred in 2 h. As $3K^+$ $TSPTM^{3-}$ is in large excess with respect to poly(phenol), it is assumed that the deprotonated form of poly(phenols) **2**, **4**, and **6** is the only reducing species present in the medium at pH 8 reacting with $3K^+$ $TSPTM^{3-}$ in the first and rapid stage of the reaction. Therefore, the high value of the rate constant k_1 for **2**, **4**, and **6** must correspond to the transfer of the first electron from the poly(phenol) in its deprotonated form. This first stage of the reaction at pH 8 is much slower for catechol and is so slow in (−)-epicatechin (**3**) that virtually merges with the succeeding processes without any difference in the slope of the kinetics graph, such that the value of the rate constant does not corresponds to an elementary process but to a complex process in which 3.6 electrons are transferred.

An analysis of the graphics of kinetics of poly(phenols) **2**, **4**, and **6** with $3K^+$ $TSPTM^{3-}$ at pH 7 indicates that the decrease of the absorbance of the band of $TSPTM^{3-}$ at λ=385 nm is more

uniform, and a first well defined first electron transfer cannot be distinguished from the rest. The kinetics of the reaction can be treated as a multistep process with several electron transfers in the first 60 min of reaction. Surprisingly, the stoichiometric value in 2 h of reaction is greater than in the reactions at pH 8. Regarding the reaction of $3K^+$ $TSPTM^{3-}$ with catechol (**1**) and (−)-epicatechin (**3**), the very low stoichiometric value in 2 h of reaction, approaching only the value of one electron transfer, is a consequence of the slowness of the reactions. In both cases, $[ArO^{\bullet}] \ll [ArOH]$ in buffer solution at pH 7.

Regarding the reduction reaction of $3K^+$ $TSPTM^{3-}$ with quercetin as antioxidant, experiments at pH 7 could not be carried out because the poly(phenol) was not soluble, and kinetic analysis of experiments at pH 8 (Fig. 3) showed moderate reaction rates. A first elemental process of one electron transfer is not distinguished in the reaction of quercetin with $3K^+$ $TSPTM^{3-}$ and the multistep process during the first 24 min is due to the transfer of a non-integer number of electrons (2.57). The total stoichiometric value in 2 h time corresponds to practically 4 electrons transfer. Now, $[ArO^-] > [ArOH]$ as indicated by the pK_a of the quercetin.

Each SO_3H function in the α*H*-TSPTM behaves as a strong acid and should be completely ionized at pH 7–8. However, as it concerns to the labile hydrogen of the central carbon atom, it is completely associated at pH 7–8, and even at pH = 10. The UV–vis spectrum of the completely associated trianion α*H*-$TSPTM^{3-}$ (Scheme 2) in saturated aqueous solution of $NaHCO_3$ (pH = 9) and the trianion α*H*-$TSPTM^{3-}$ in equilibrium with its anionic species, the tetraanion $TSPTM^{4-}$ (Scheme 2), in buffer solution at pH = 12.6 are showed in Fig. 4. The experimental acid dissociation constant of the CH deprotonation of α*H*-TSPTM determined spectrophotometrically was, $pK_a = 13$.

The radical-scavenging activity of poly(phenols) **1**–**6** against $3K^+$ $TSPTM^{3-}$ in aqueous buffer solutions (pH 7 and 8) involves an electron transfer from the antioxidant to the radical to generate the tetraanion $TSPTM^{4-}$. However, as this tetraanion is the strong conjugated base of a very weak acid, it is quickly protonated to give the trianion α*H*-$TSPTM^{3-}$, so that it is not detected by UV–vis spectroscopy in many of these reducing reactions. The tetraanion is clearly seen in the first fast stages of the experiments of $3K^+$ $TSPTM^{3-}$ with pyrogallol and (−)-epigallocatechin at pH 8, confirming the electron transfer mechanism of these processes (Scheme 4). Fig. 5 shows the evolution of the UV–vis spectrum in the early stages of the reaction of $3K^+$ $TSPTM^{3-}$ with pyrogallol at pH 8 showing the strong absorption of the tetraanion at $\lambda = 535$ nm and its decrease with time. This reductive process to the tetraanion is followed by a neutralization process to α*H*-$TSPTM^{3-}$. The detection of the tetraanion in the UV–vis spectrum suggests that the neutralization rate of the anion is slower than the reduction rate of $TSPTM^{3-}$.

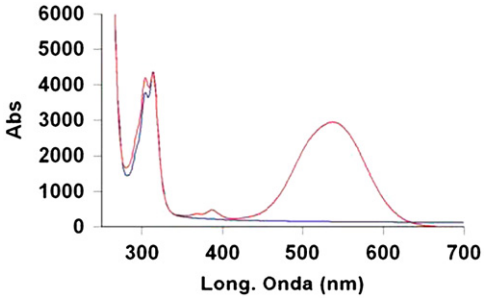


Fig. 4. UV–vis spectra of α*H*-TSPTM; (black band) in saturated $NaHCO_3$ aqueous solution (pH = 9) and (red band) in aqueous buffer solution (pH = 12.6). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Conclusions

The TSPTM tri-potassium salt, $3K^+$ $TSPTM^{3-}$, is a good chemical sensor of the radical-scavenging activity of poly(phenols) in aqueous media. The water solubility of this new radical species with neutral hydrolysis and its stability at all pH values, from acid to basic solutions, facilitates the study of the influence of the pH of the medium on the activity of poly(phenols). Therefore, we have measured the radical-scavenging activity of three flavonoids of the catechol and pyrogallol type and the activity of catechol, pyrogallol and methyl gallate at pH values 7 and 8 which are close to physiological pH value (7.4). TSPTM tripotassium salt is therefore valuable in determining relative antioxidant activity

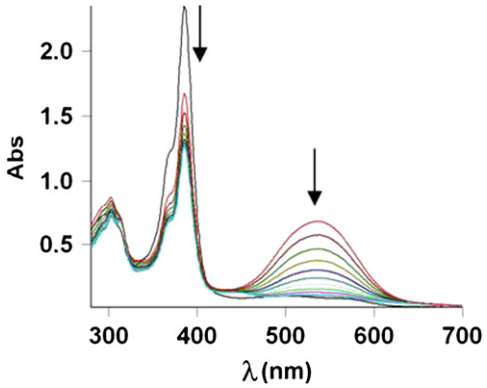
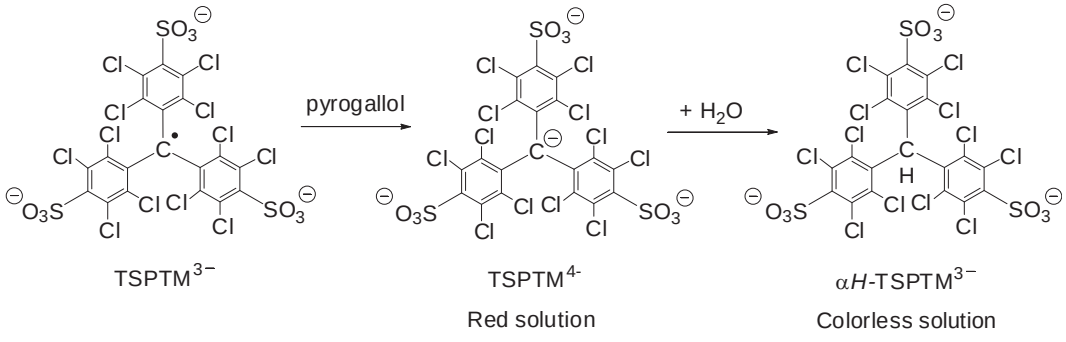


Fig. 5. Evolution of the UV–vis spectra of a aqueous buffer solution (pH = 8) of the $3K^+$ $TSPTM^{3-}$ and pyrogallol (10:1). The black band corresponds to the blank solution; the red one corresponds to the reaction after a time ($t \leq 1$ min) of mixing the reagents; evolution of the spectra ($t = 3$ min) in the successive bands. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Scheme 4.

in biological systems. Another characteristic of this radical species is that it reacts exclusively by an electron transfer mechanism against the reducing poly(phenols) due to its electron-accepting properties to generate the tetraanion $TSPTM^{4-}$. Therefore, the experiments to determine the radical-scavenging activity of poly(phenols) with $3K^+$ $TSPTM^{3-}$ give a measure of the electron-donor properties of polyphenols which are related to their ionization potentials (IP). As IP is a function of the pH of the medium, the electron donor ability of a poly(phenol) depends on the acidity or basicity of the fluid in question. The radical-scavenging activity of poly(phenols) **1–6** is higher at pH 8 than at pH 7, as shows the k_1 value of the first redox process in Table 1. It is evident from the values of k_1 and total stoichiometric (TS) factors of (poly)phenols 1–4 in Table 1 that $3K^+$ $TSPTM^{3-}$ is able to discriminate between catecholic and pyrogallolic moieties of poly(phenols) in water solutions by taking measurements at different pH values. $3K^+$ $TSPTM^{3-}$ provides a convenient and accurate means of evaluating the electron transfer capacity of (poly)phenols in biologically significant aqueous environments. By adjusting the pH of the medium $3K^+$ $TSPTM^{3-}$ will easily detect those moieties (e.g. pyrogallol) most prone to elicit prooxidant effects.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.09.010.

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