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Can aluminum, a non-redox metal, alter the thermodynamics of key biological redox processes? The DPPH-QH_2 radical scavenging reaction as a test case

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ABSTRACT

The increased bioavailability of aluminum has led to a concern about its toxicity on living systems. Among the most important toxic effects, it has been proven that aluminum increases oxidative stress in biological systems, a controversial fact, however, due to its non-redox nature. In the present work, we characterize in detail how aluminum can alter redox equilibriums by analyzing its effects on the thermodynamics of the redox scavenging reaction between DPPH·, a radical compound often used as a reactive oxygen species model, and hydroquinones, a potent natural antioxidant. For the first time, theoretical and experimental redox potentials within aluminum biochemistry are directly compared. Our results fully agree with experimental reduction and oxidation potentials, unequivocally revealing how aluminum alters the spontaneity of the reaction by stabilizing the reduction of DPPH· to $DPPH^-$ and promoting a proton transfer to the diazine moiety, leading to the production of a DPPH-H species. The capability of aluminum to modify redox potentials shown here confirms previous experimental findings on the role of aluminum to interfere with free radical scavenging reactions, affecting the natural redox processes of living organisms.

1. Introduction

The massive industrial use of aluminum, the most abundant metal in the Earth's crust, has implied its introduction into the biosphere, contrary to previous natural conditions in which complex geochemical cycles prevented its solubilization [1,2]. Consequently, humans are nowadays highly exposed to this metal, a fact linked to various diseases, starting from early evidence of dialysis encephalopathy or osteodystrophy in patients with renal failure under dialysis treatment [3] to more recent evidence linking aluminum to several neurodegenerative disorders [4]. However, the molecular bases for these toxic effects are still not well understood, partially due to the inherent difficulties of interpreting experimental data. In this sense, theoretical calculations have become fundamental to get insight into the bioinorganic chemistry of aluminum and its potentially harmful effects [5–13].

The pro-oxidant ability of aluminum is one of its most known toxic effects [10–12,14], a factor frequently underestimated due to its non-redox nature. That is, the oxidation state +3 of aluminum in a biological system is maintained unaltered in different biochemical environments, as demonstrated recently [15]. However, this does not imply that aluminum can not alter important redox cycles [16] in vitro and in vivo. The early hypothesis of the possibility of forming an aluminum-superoxide complex [14], which would augment the lifetime of this radical species, has been proven computationally [8,10,12]. The thermodynamic stabilization of a metal-superoxide complex is not by itself explanatory of the mechanism behind the pro-oxidant ability of aluminum. However, Fukuzumi et al. [17–19] have reported a linear relationship between the strength of a metal–superoxide interaction and its oxidant activity of a metal. Despite these circumstantial pieces of evidence that point to aluminum as a promoter of oxidative stress, the

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topic is still highly controversial due to the lack of direct measures of the effect of aluminum in the redox potentials of radical species.

In this context, the recent work by Nakanishi et al. [20] is highly relevant. They investigated the promotion of the radical scavenging reaction of hydroquinone derivatives (Me_nQH_2, n=0–4) by aluminum ion, using 2,2–diphenyl–1–picrylhydrazyl (DPPH·) as a model for a peroxyl radical. The authors were able to determine the reduction/oxidation potentials of DPPH·/Me_nQH_2 in the absence and presence of aluminum, concluding unequivocally that Al(III) enhances the electron-accepting ability of DPPH· and consequently accelerates the DPPH· scavenging reaction by hydroquinones. This supported previous studies where it was established that radical scavenging reactions can be affected by redox inactive metals, e.g., Mg(II), Al(III) and Sc(III) [21–30].

In the present work, we give computational support to this study, determining how the presence of aluminum can alter the oxidation/reduction potential of these species. To achieve our goal, we investigated the different coordination modes that aluminum can form with DPPH· and QH_2 mimicking the experimental conditions and assuming an outer-sphere mechanism, as done in the reference experimental study [20]. Our results show a good agreement with experiments, and they allow us to identify the key species involved in promoting the redox reaction by aluminum. This gives valuable insights into the properties of aluminum that favor the electron transfer from hydroquinones to the 2,2–diphenyl–1–picrylhydrazyl radical, allowing us to identify unambiguously aluminum as a potential factor that alters the redox potentials of these species. Thus, our results provide a solid ground to identify Al^{3+} as a possible toxic factor in biological media by affecting the thermodynamics of essential redox reactions, despite the non-redox nature of this metal.

2. Computational details

All structures were optimized and characterized using Gaussian16 software [31] employing the M06-2X density functional [32] in conjunction with the 6-31+G(d) basis set for all atoms [33–40] and taking into account ethanol solvation effects using the *Polarizable Continuum Model* (PCM) approach [41] (hereinafter referred to as L1 level of theory). The characterization of optimized structures (at 298.15K and 1atm.) confirmed all minima have no imaginary frequencies.

Electronic and solvation energies were further refined by single-point calculations at the M06-2X/6-311++G(3df, 2p)/PCM(ethanol) level of theory [33,37,39,40,42,43] (hereinafter referred to as L2 level of theory). Final free energies reported in this study also take into account the free energy change associated with moving from a standard-state gas phase of 1 atm of pressure to gas-phase state with specific concentrations ($\Delta G^{o/*}$). Then, the total Gibbs energy (G) is given by:

$$G = E_{\mathbf{L2}} + G_{solv(\mathbf{L2})} + G_{corr(\mathbf{L1})} + \Delta G^{o/*} \quad (1)$$

The concentrations used to estimate $\Delta G^{o/*}$ are in agreement with the experimental conditions described in Ref. [20] in order the results to be comparable. Thereby, the following gas-phase concentrations were used: 5.54 M for water, 15.36 M for ethanol, 2.4×10^{-3} M for QH_2 and its derivatives, 2.0×10^{-3} M for DPPH· and its derivatives, 0.2 M for Al^{3+} and its derivatives, and different proton concentrations according to the pH in each situation. Limiting reagent's concentration is the one that defines the concentration of any formed intermediate specie. See Supporting Information (SI) for further details.

Reduction potentials reported are relative to the saturated calomel electrode (SCE), in agreement with the experimental study, and satisfy the following expression:

$$E = -\frac{\Delta G}{nF} - E^{o}_{SCE} \quad (2)$$

were ΔG is the free energy change associated with the reduction process at the specific conditions studied, n is the number of electrons involved in the redox reaction, F is the Faraday constant, and E^{o}_{SCE} is the absolute reduction potential of the reference calomel electrode, which has a value of +4.521eV [44]. See SI for further details.

3. Results and discussion

3.1. Experimentally determined redox behavior

The one-electron redox potentials of QH_2, Me_4QH_2, and DPPH· (Fig. 1) with and without the presence of the Al^{3+} ion in solution were studied computationally and compared with the experimental redox potentials presented in Ref. [20] in order to validate the level of theory and results. Experimental values are summarized in Table 1.

Considering the contrary relation between redox potentials and free energies (E = -ΔG/nF), experimental results exhibit that the one-electron donating ability of hydroquinones decreases with the presence of Al^{3+}, while the electron-accepting ability of DPPH· increases. However, the reduction of DPPH· is more promoted rather than the oxidation of hydroquinones impeded, and as a consequence, the global redox DPPH· radical scavenging reaction of hydroquinones in the hydroalcoholic medium evolves from non-spontaneous ($E_{reac} < 0$) to spontaneous ($E_{reac} > 0$) by the presence of Al^{3+}.

3.2. Theoretical redox potentials in the absence of Al^{3+}

Table 2 brings together the computed (and reference) reduction and oxidation potentials for hydroquinones and DPPH· without Al^{3+}'s presence in EtOH–H_2O (9:1 v/v) solutions. PH values of 5.5, 4.6 and 7.5 are used to compute proton coupled electron transfer (PCET) potentials for hydroquinone, tetramethylhydroquinone, and DPPH·, respectively. For hydroquinones (simple and tetramethylated), the used pH values were in agreement with the experimental ones [20]. Taking into account that the pK_a values of the studied hydroquinones in aqueous solution are in the range of 9.9–11.3 units [20], which are significantly higher than the pH values of the experiment, and that the presence of ethanol in water-organic solvent mixtures increases pK_a values [45], it is reasonable to assume that the reduced species of hydroquinone's oxidation reactions correspond to their neutral forms (QH_2 and Me_4QH_2) and not to the deprotonated ones (QH^- and Me_4QH^-). For DPPH·, the experimental pH value of the EtOH–H_2O (9:1 v/v) solution is not reported. The value of 7.5 has been assumed in agreement with similar studies were DPPH· is used as oxidant in radical scavenging essays, where typically buffers are chosen to guarantee pH values between 4.6 and 7.4 units [46,47]. Taking into account that DPPH-H presents a pK_a value of 8.6 in EtOH–H_2O (1:1 v/v) solutions [47], the protonated form (DPPH-H) is expected to be the reduced species present in solution. Nevertheless, the $DPPH^-$ form has also been considered in the study of DPPH·'s reduction potentials.

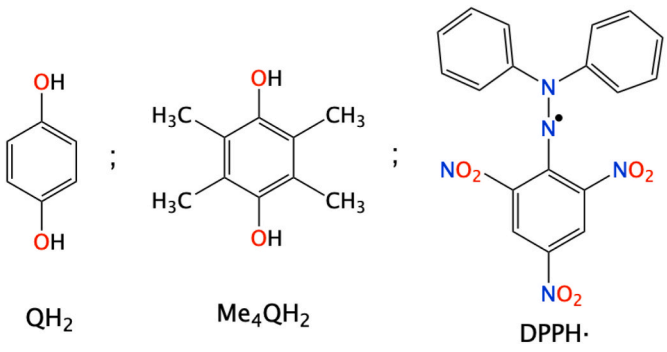

Fig. 1. Structures of the QH_2, Me_4QH_2, and DPPH· redox species studied in this work.

Table 1
Oxidation potential (E_{ox}) values of hydroquinones and reduction potential (E_{red}) values of DPPH· in EtOH–H_2O (9:1 v/v) in the presence and absence of $Al(ClO_4)_3$. Redox potentials relative to SCE (data from Ref. [20]), following the sign convention where half-reactions are written with products on the right. $E_{reac} = E_{ox} + E_{red}$. All quantities are in V.

$[Al(ClO_4)_3]/M$	E_{ox}		E_{red}	E_{reac}
	QH_2	Me_4QH_2	DPPH·	QH_2 + DPPH·
0.0	−0.39	−0.19	+0.23	−0.16
0.2	−0.55	−0.28	+0.70	+0.15

Table 2
Theoretical oxidation (E_{ox}) and reduction potentials (E_{red}) for hydroquinones and DPPH·, respectively, in the absence of aluminum. All values are in V.

	E_{red}/E_{ox}
QH_2	
a. $QH_2 \rightarrow QH_2^+ + e^-$	−1.41
b. $QH_2 \rightarrow QH\cdot + e^- + H^+$	−0.40
Exp Ref.	−0.39
DPPH·	
c. $DPPH\cdot + e^- \rightarrow DPPH^-$	+0.27
d. $DPPH\cdot + H^+ + e^- \rightarrow DPPHH$	+0.32
Exp Ref.	+0.23
Me_4QH_2	
e. $Me_4QH_2 \rightarrow Me_4QH_2^+ + e^-$	−1.12
f. $Me_4QH_2 \rightarrow Me_4QH\cdot + e^- + H^+$	−0.36
Exp Ref.	−0.19

Both electron transfer (ET) and PCET oxidation and reduction processes were analyzed. For hydroquinones (*i.e.* QH_2 and Me_4QH_2), the computed oxidation potentials of PCET reactions are clearly the ones that agree with experimental values (reactions b and f). E_{ox}(reac. b) highly matches with the experimental value and E_{ox}(reac. f) differs 0.17 V from the experiment, which lies in the accepted error range. Overall, this allow us to establish that the one-electron oxidation processes measured for hydroquinones are PCET. In contrast, the assignation is unclear for DPPH· because both ET and PCET computed reduction potentials have values close to the experiment, differing up to 0.09 V.

Finally, we can analyze the effect of methylation in hydroxyquinones. Methyl groups are inductive electron-donating groups, and therefore, methylation enriches the electron density of hydroquinones making the system more nucleophilic and stabilizing the oxidized forms. As a result of the stabilization, oxidation and deprotonation reactions are less endergonic and their respective oxidation potentials less negative. This tendency, which clearly comes out in experimental data, is also qualitatively picked up in our calculations. Methylated hydroquinone exhibits shifts in the oxidation potentials with respect to the non-methylated case of +0.29 V and +0.04 V for the ET and PCET processes, respectively. These shifts are comparable with the experimental one of +0.20 V.

3.3. Theoretical redox potentials in the presence of Al^{3+}

When Al^{3+} is present in solution, different coordination complexes with solvent molecules and the redox species (*i.e.* DPPH· and QH_2) can be formed. Apart from the formation of organometallic complexes with a positive trivalent charge, aluminum, as a strong Lewis acid, can reduce the pKa of the coordinated ligands [9], and prompt their deprotonation and the formation of coordination complexes of lower charge.

Table 3 collects the relative stabilities (ΔG) of all the trivalent, divalent, and monovalent most-stable stereoisomers of Al^{3+}-EtOH, Al^{3+}-QH_2, Al^{3+}-Me_4QH_2, and Al^{3+}-DPPH· complexes that have been considered as possible thermodynamic species that may undergo further oxidation or reduction reactions (Fig. 2). SI includes, when applicable, diastereoisomers cis/trans for relative EtO^-/redox-specie orientations (Tables S3–S5). Both pH=1.5 and pH=5.5 are examined in order to reproduce experimental solution conditions (EtOH–H_2O (9:1 v/v)) of hydroquinones and DPPH·, respectively.

Table 3
Relative stabilities (Δ*G*) of the studied Al^{3+} complexes at different pH values in kcal/mol.

Main Al^{3+} complex[a]	pH	
	1.5	5.5
$[Al(EtOH)_6]^{3+}$	0.0	0.0
$[Al(EtOH)_5(QH_2)]^{3+}$	11.8	11.8
$[Al(EtOH)_5(Me_4QH_2)]^{3+}$	15.0	15.0
$[Al(EtOH)_5(DPPH\cdot)]^{3+}$	9.1	9.1
$[Al(EtOH)_5(EtO^-)]^{2+}$	8.0	−4.0
$[Al(EtOH)_5(QH^-)]^{2+}$	8.7	−0.7
$[Al(EtOH)_5(Me_4QH^-)]^{2+}$	10.8	1.4
$[Al(EtOH)_4(EtO^-)(QH_2)]^{2+}$	18.4	9.0
$[Al(EtOH)_4(EtO^-)(Me_4QH_2)]^{2+}$	19.8	10.4
$[Al(EtOH)_4(EtO^-)(DPPH\cdot)]^{2+}$	15.8	6.5
$[Al(EtOH)_4(EtO^-)_2]^{+}$	27.7	10.2

[a] The column just displays the main Al^{3+} complex involved in each mass balanced equation. In this way, $[Al(EtOH)_6]^{3+}$ is the main Al^{3+} complex of the reference reagents, *i.e.* $[Al(EtOH)_6]^{3+} + 2\times(H_2O) + QH_2 + Me_4QH_2 + DPPH\cdot$. In turn, $[Al(EtOH)_5(QH_2)]^{3+}$ refers to the Gibbs energy difference between $[Al(EtOH)_5(QH_2)]^{3+} + 2\times(H_2O) + EtOH + Me_4QH_2 + DPPH\cdot -$ (the general reagents); and so on. Waters have been included in order to treat the deprotonation of the compounds.

The good description of the relative stabilities of the Al^{3+} complexes included in Table 3 is not straightforward, as it has been already reported for aqueous media and $[Al(H_2O)_6]^{3+}$ species [9,48]. The inclusion of explicit solvent molecules describing up to the second solvation sphere of the complex was revealed necessary to properly describe Al^{3+}-water interactions. Specifically, the explicit description of hydrogen-bond nets that solvent molecules and ligands form in aqueous or hydroalcoholic media seems necessary to successfully determine the corresponding pKa values of these Al^{3+} complexes and to properly estimate their relative energies between protonated and deprotonated forms. However, for practical reasons, we have not considered additional explicit solvation spheres here and the solvent is just described using the PCM approach. Then, redox potentials of hydroquinones and DPPH· Al^{3+} complexes that lie within a ΔG confidence interval of 10 kcal/mol are analyzed.

Hydroquinone, QH_2. To analyze the E_{ox} values of hydroquinones (pH = 1.5) we have considered three possibilities as initial thermodynamic species: i) $[Al(EtOH)_6]^{3+} + QH_2$, ii) $[Al(EtOH)_5(EtO^-)]^{2+} + QH_2$, and iii) $[Al(EtOH)_5(QH^-)]^{2+}$. Table 4 summarizes the computed oxidation potentials and the oxidation-potential changes ($\Delta E_{ox}^{\varnothing\rightarrow Al}$) from the aluminum-free situation (reaction b in Table 2).

Oxidation reactions that exhibit potential energy values consistent with experimental results are Q2, Q7, and Q9. Each reaction evolves from a different thermodynamic species; *i.e.*, i) $[Al(EtOH)_6]^{3+} + QH_2$, ii) $[Al(EtOH)_5(EtO^-)]^{2+} + QH_2$, and iii) $[Al(EtOH)_5(QH^-)]^{2+}$, respectively. Oxidation reactions with higher negative oxidation potentials than Q2, Q7, and Q9 reactions entail more endergonic processes than these where unstable species are formed and, consequently, they can be automatically discarded as possible hydroquinone's oxidation reactions in solution with the presence of Al^{3+}. Q4 and Q10 reactions deserve further comments because entail exergonic and *spontaneous* processes that should be preferred over endergonic ones. However, further remarks can be made taking into account the experimental conditions.

Considering the acidic experimental conditions (pH = 1.5), a further justification of the most likely hydroquinone's oxidation process in the presence of Al^{3+} can be done. Despite in Q10 we have considered reagent $[Al(EtOH)_5(QH^-)]^{2+}$, it can not be formed directly by

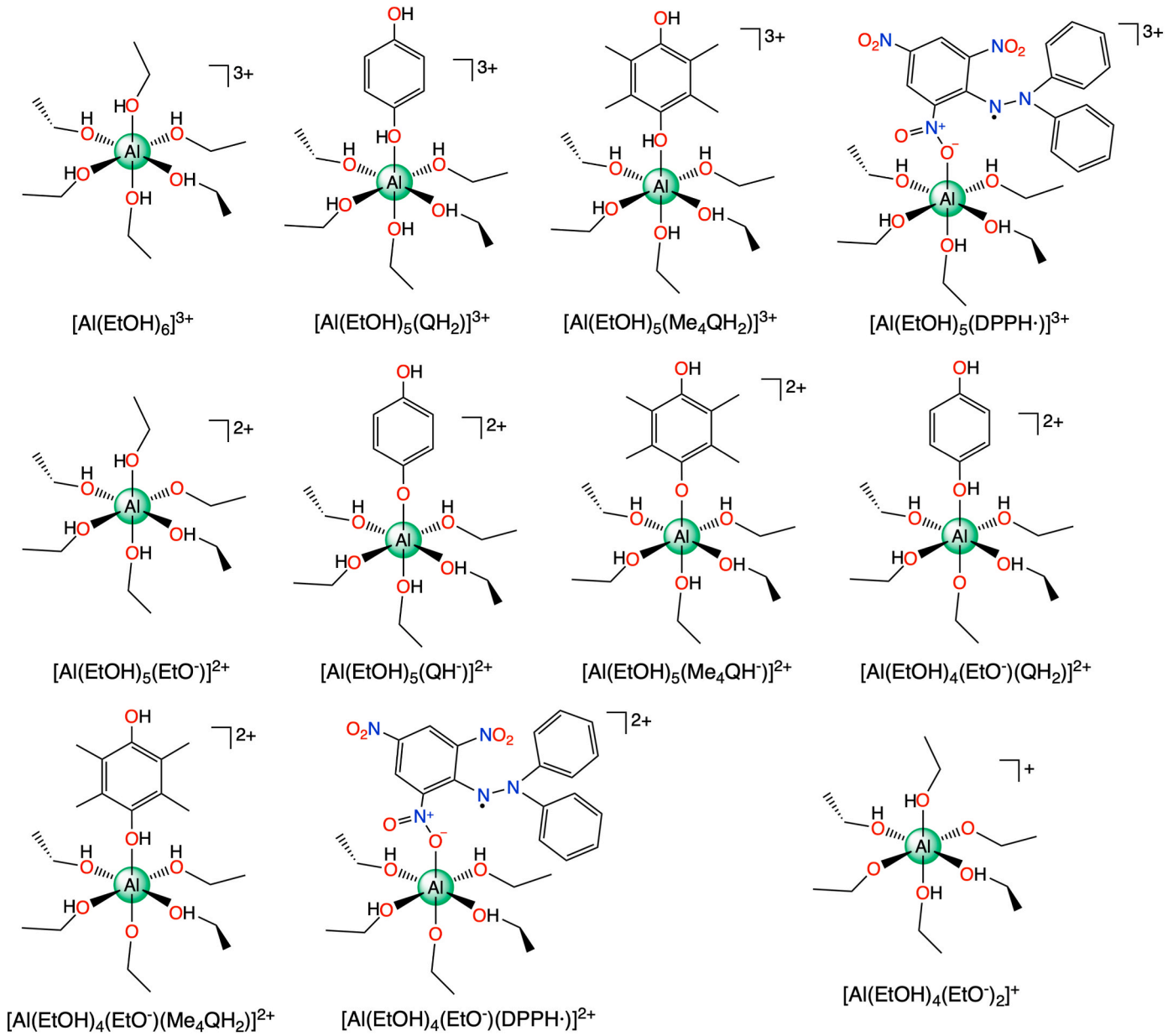

Fig. 2. Trivalent, divalent, and monovalent Al^{3+} complexes considered in this study.

$[Al(EtOH)_6]^{3+} + QH^-$ because at pH = 1.5 deprotonated hydroquinones are not present in solution. Instead, it may come from the deprotonation of other Al^{3+} complex such as $[Al(EtOH)_5(QH_2)]^{3+}$ through a multi-step process as it is shown in Fig. S1a). $[Al(EtOH)_5(QH_2)]^{3+}$ is a high energy complex (ΔG of 11.8 kcal/mol, reported in Table 3), which acts as an intermediate preventing the formation of $[Al(EtOH)_5(QH^-)]^{2+}$ and precluding reactions Q8, Q9, and Q10 to be feasible oxidation processes. A similar situation may happen for Q4. Q4 reaction entails two unfavourable processes (ethanol deprotonation and hydroquinone PCET), which highly imply the formation of an endergonic intermediate through a multi-step reaction mechanism (like the ones of Q3 and Q1) (see Fig. S1b). The high energy necessary to form these intermediates prevents the formation of $[Al(EtOH)_4(EtO^-)(QH\cdot)]^{2+}$ through them.

In summary, our computational study reveals that reactions Q2 and Q7 describe the most likely hydroquinone's oxidation reactions for the given experimental conditions in solution and with the presence of Al^{3+}. They differ −0.07 and −0.01 V with the experimental reference, respectively, and, energetically, both results are equiprobable. However, given the acidic experimental conditions (pH = 1.5), $[Al(EtOH)_6]^{3+}$ is a more likely populated species than $[Al(EtOH)_5EtO^-]^{2+}$, and therefore, Q2 should be considered the preferred oxidation process. Both reactions entail a PCET process; which is the same oxidation process than the one observed without the presence of Al^{3+} ions in solution (b in Table 2). If we compare oxidation potentials reported in Table 4 with E_{ox}(b) of Table 2, the oxidation potential shift due to the presence of aluminum ($\Delta E_{ox}^{\varnothing \rightarrow Al}$) can be studied. We can observe that when Al^{3+} is present, more negative results are obtained. This trend is also observed experimentally (Table 4) and our results replicate it quantitatively. Then, we can conclude that QH_2 oxidation reactions are more endergonic and unfavored when Al^{3+} ion is present in solution. The reason for this increase in endergonicity is a balance between the promotion of proton release and the disfavor of electron lose in the presence of aluminum. Thus, on one hand, aluminum has a tendency to favor deprotonation of the ligands that are attached to it, so that it can interact with a negatively charged species; however, for the same reason, it tends to disfavor the lose of negative charge of the ligands, since this implies a decrease of the

Table 4
Oxidation potentials, in V, of QH_2 assisted by different Al^{3+} complexes at pH = 1.5 and relative reduction potential with respect to the values in the absence of aluminum, $\Delta E_{ox}^{\varnothing\rightarrow Al}$.

Oxidation reactions	E_{ox}	$\Delta E_{ox}^{\varnothing\rightarrow Al}$
Q1. $[Al(EtOH)_6]^{3+} + QH_2 \rightarrow [Al(EtOH)_5(QH_2^+)]^{4+} + e^- + EtOH$	−3.35	−2.96
Q2. $[Al(EtOH)_6]^{3+} + QH_2 \rightarrow [Al(EtOH)_5(QH\cdot)]^{3+} + e^- + H^+ + EtOH$	−0.62	−0.22
Q3. $[Al(EtOH)_6]^{3+} + QH_2 \rightarrow [Al(EtOH)_4(EtO^-)(QH_2^+)]^{3+} + e^- + H^+ + EtOH$	−1.65	−1.26
Q4. $[Al(EtOH)_6]^{3+} + QH_2 \rightarrow [Al(EtOH)_4(EtO^-)(QH\cdot)]^{2+} + e^- + 2H^+ + EtOH$	0.58	0.98
Q5. $[Al(EtOH)_5(EtO^-)]^{2+} + QH_2 \rightarrow [Al(EtOH)_4(EtO^-)(QH_2^+)]^{3+} + e^- + EtOH$	−2.79	−2.40
Q6a. $[Al(EtOH)_5(EtO^-)]^{2+} + QH_2 \rightarrow [Al(EtOH)_5(QH\cdot)]^{3+} + e^- + H^+ + EtO^-$	−3.58	−3.19
Q6b. $[Al(EtOH)_5(EtO^-)]^{2+} + QH_2 \rightarrow [Al(EtOH)_5(QH\cdot)]^{3+} + e^- + EtOH$	−1.76	−1.37
Q7. $[Al(EtOH)_5(EtO^-)]^{2+} + QH_2 \rightarrow [Al(EtOH)_4(EtO^-)(QH\cdot)]^{2+} + e^- + H^+ + EtOH$	−0.56	−0.16
Q8. $[Al(EtOH)_5(QH^-)]^{2+} \rightarrow [Al(EtOH)_5(QH\cdot)]^{3+} + e^-$	−1.68	−1.29
Q9. $[Al(EtOH)_5(QH^-)]^{2+} \rightarrow [Al(EtOH)_4(EtO^-)(QH\cdot)]^{2+} + e^- + H^+$	−0.49	−0.09
Q10. $[Al(EtOH)_5(QH^-)]^{2+} \rightarrow [Al(EtOH)_5(EtO^-)]^{3+} + QH\cdot + H^+ + e^-$	11.12	11.52
Exp. ref.	−0.55	−0.16

electrostatic interaction between the ligand and aluminum. In PCET processes, where both reactions coexist, the lose of an electron has a higher energetic cost than the lose of a proton in these aluminum complexes, and the overall effect is a more negative oxidation potential in the presence of aluminum. Notice for example that in the case of Q4 where one electron is released but two protons are unbound instead of one, the end-result is an exergonic process.

Tetramethylhydroquinone, Me_4QH_2. Table 5 reports the oxidation potentials determined for Me_4QH_2 when Al^{3+} is present in solution at a 1.5 pH value. Assuming that the oxidation process will evolve following the same trend as for QH_2, only the key oxidation reactions determined before (*i.e.*, Q2, Q7, and Q9) have been studied here. However, as discussed before, the existence of the reagent $[Al(EtOH)_5(Me_4QH^-)]^{2+}$ of reaction MeQ9 is less likely than the existence of the other Al^{3+} reagents involved in MeQ2 and MeQ7 reactions. The $[Al(EtOH)_5(Me_4QH^-)]^{2+}$ reagent complex of MeQ9 is more unstable (ΔG of 10.8 kcal/mol) than the equivalent non-methylated form $[Al(EtOH)_5(QH^-)]^{2+}$ (ΔG of 8.7 kcal/mol) compared with the most stable form, $[Al(EtOH)_6]^{3+}$, at pH=1.5 (Table 3). Moreover, it can neither be formed directly by $[Al(EtOH)_6]^{3+} + Me_4QH^-$ because the deprotonated form of Me_4QH_2 is not present in solutions at pH = 1.5 as its pKa value is > 11 [20]. Overall, we consider that MeQ2 and MeQ7 are the oxidation reactions of Me_4QH_2 that better represent experimental results. Then, as for the QH_2 case, the most likely oxidation processes of Me_4QH_2, comparing to the experimental oxidation potential, are dehydrogenation reactions (PCET reactions) that also imply the coordination of Me_4QH_2 with the Al^{3+} complex.

Table 5
Oxidation potentials, in V, of Me_4QH_2 assisted by different Al^{3+} complexes at pH = 1.5. Only key reactions have been considered. Oxidation potential differences with respect to the absence of aluminum, $\Delta E_{ox}^{\varnothing\rightarrow Al}$, and the non-methylated quinone, ΔE_{ox}^{Met}, are reported.

Oxidation reactions	E_{ox}	$\Delta E_{ox}^{\varnothing\rightarrow Al}$	ΔE_{ox}^{Met}
MeQ2. $[Al(EtOH)_6]^{3+} + Me_4QH_2 \rightarrow [Al(EtOH)_5(Me_4QH\cdot)]^{3+} + e^- + H^+ + EtOH$	−0.39	−0.03	0.23
MeQ7. $[Al(EtOH)_5(EtO^-)]^{2+} + Me_4QH_2 \rightarrow [Al(EtOH)_4(EtO^-)(Me_4QH\cdot)]^{2+} + e^- + H^+ + EtOH$	−0.46	−0.10	0.10
MeQ9. $[Al(EtOH)_5(Me_4QH^-)]^{2+} \rightarrow [Al(EtOH)_4(EtO^-)(Me_4QH\cdot)]^{2+} + e^- + H^+$	−0.30	0.06	0.19
Exp. Ref.	−0.28	−0.09	0.27

Calculated oxidation potentials of Me_4QH_2 in the presence of Al^{3+} differ between 0.11 and 0.18 V from the experimental E_{ox} of −0.28 V. The deviation from the experimental value is moderate but acceptable. However, we would like to remember that calculated oxidation potentials of Me_4QH_2 without considering Al^{3+} presence already exhibited a moderate deviation from the experimental value. In fact, regarding the shift of the oxidation potentials when aluminum is considered ($\Delta E_{ox}^{\varnothing\rightarrow Al}$) or upon methylation ΔE_{ox}^{Met}, the agreement with experimental figures is very reasonable. In this sense, our calculations replicate the experimental trend that Al^{3+} disfavors Me_4QH_2 oxidation (i.e., negative ($\Delta E_{ox}^{\varnothing\rightarrow Al}$), and that methylation favors oxidation (positive values of ΔE_{ox}^{Met}).

DDPH·. Taking into account precedent experimental studies [46], the one-electron reduction potential of DPPH· in the presence of Al^{3+} has been studied here considering a solution with a pH value of 5.5. As it happened for hydroquinones, the presence of Al^{3+} reduces the original pH of the solution without Al^{3+} (for the DPPH· case the reduction goes from 7.5 to 5.5 pH units). Globally, the pH of the DPPH· solution with the presence of Al^{3+} is more basic than the pH of equivalent hydroquinone solutions and this has an effect in the relative stability of the Al^{3+} complexes that may be formed. As it can be observed in Table 3, when the solution becomes more basic, an extra stabilization of less charged complexes is achieved. We have considered $[Al(EtOH)_5(EtO^-)]^{2+}$, $[Al(EtOH)_6]^{3+}$, $[Al(EtOH)_4(EtO^-)(DPPH\cdot)]^{2+}$, and $[Al(EtOH)_5(DPPH\cdot)]^{3+}$ complexes to study one-electron reduction potentials of DPPH·. It is important to note that for this situation (pH = 5.5) the most stable Al^{3+}-complexes are divalent $[Al(EtOH)_5(EtO^-)]^{2+}$ and $[Al(EtOH)_4(EtO^-)(DPPH\cdot)]^{2+}$ ones, instead of $[Al(EtOH)_6]^{3+}$ and $[Al(EtOH)_5(DPPH\cdot)]^{3+}$. This could be expected from the chelation of alcohol ligands to aluminum, which causes important shifts in their pKa values [9].

Table 6 reports all the one-electron reduction potentials analyzed for DPPH· considering Al^{3+} present in solution. In all Al^{3+}-complexes where DPPH· is coordinated to Al^{3+}, the coordination is done through an orto nitro group of the picryl substituent of DPPH·, kO (Fig. 3), because this coordination mode is always more stable than the diazane coordination, kN (see Table S2).

One-electron reduction potentials reported in Table 6 that exhibit a better agreement with experimental potential values are those from D5a, D5b, D7 and D12 reduction reactions. All these reactions except D12 derive from the initial $[Al(EtOH)_5(EtO^-)]^{2+}$ form, while D12 reduction reaction emanates from $[Al(EtOH)_4(EtO^-)(DPPH\cdot]^{2+}$. As the $[Al(EtOH)_5(EtO^-)]^{2+}$ form has been characterized as the most stable ethanol-solvated Al^{3+}-complex at pH = 5.5, being ΔG = 10.5 kcal/mol more favorable than the $[Al(EtOH)_4(EtO^-)(DPPH\cdot]^{2+}$ complex; the presence of $[Al(EtOH)_4(EtO^-)(DPPH\cdot]^{2+}$ in solution can be almost neglected and we are not going to consider D12 as a feasible option. Table 6 includes several redox reactions of DPPH· that present higher positive E_{red} values than D12. Despite their E_{red} values imply these reactions are more exergonic and thermodynamically favoured than the ones previously proposed, they were unfeasible due to kinetics or the thermodynamics of the initial aluminum form. They involve the $[Al(EtOH)_5(DPPH\cdot)]^{3+}$, the $[Al(EtOH)_6]^{3+}$, or the $([Al(EtOH)_4(EtO^-)(DPPH\cdot)]^{2+}$ complexes, which are less likely to be

Table 6
One-electron reduction potentials, in V, of DPPH· in the presence of Al^{3+} complexes at pH = 5.5, and relative reduction potential with respect to the values in the absence of aluminum, $\Delta E_{red}^{\varnothing \rightarrow Al}$.[a]

Reduction reactions	E_{red}	$\Delta E_{red}^{\varnothing \rightarrow Al}$
D1. $[Al(EtOH)_6]^{3+} + DPPH\cdot + e^- \rightarrow [Al(EtOH)_5(DPPH^-)]^{2+} +$ EtOH	1.07	0.80
D2. $[Al(EtOH)_6]^{3+} + DPPH\cdot + e^- \rightarrow [Al(EtOH)_4(EtO^-)(DPPH^-)]^{+} + H^+ +$ EtOH	2.13	1.86
D3. $[Al(EtOH)_6]^{3+} + DPPH\cdot + H^+ + e^- \rightarrow [Al(EtOH)_5(DPPH-H)]^{3+} +$ EtOH	−1.13	−1.45
D4. $[Al(EtOH)_6]^{3+} + DPPH\cdot + H^+ + e^- \rightarrow [Al(EtOH)_4(EtO^-)(DPPH-H)]^{2+} + H^+ +$ EtOH	1.42	1.10
D5a. $[Al(EtOH)_5(EtO^-)]^{2+} + DPPH\cdot + e^- \rightarrow [Al(EtOH)_4(EtO^-)(DPPH^-)]^{+} +$ EtOH	0.51	0.24
D5b. $[Al(EtOH)_5(EtO^-)]^{2+} + DPPH\cdot + e^- \rightarrow [Al(EtOH)_4(EtO^-)(DPPH^-)]^{+} +$ EtOH	0.64	0.37
D6. $[Al(EtOH)_5(EtO^-)]^{2+} + DPPH\cdot + H^+ + e^- \rightarrow [Al(EtOH)_4(EtO^-)(DPPH-H)]^{+2} +$ EtOH	−0.19	−0.51
D7. $[Al(EtOH)_5(EtO^-)]^{2+} + DPPH\cdot + e^- \rightarrow [Al(EtOH)_3(EtO^-)_2(DPPH-H)]^{+} +$ EtOH	0.67	0.35
D8. $[Al(EtOH)_5(DPPH\cdot)]^{3+} + e^- \rightarrow [Al(EtOH)_5(DPPH^-)]^{2+}$	1.47	1.20
D9. $[Al(EtOH)_5(DPPH\cdot)]^{3+} + H^+ + e^- \rightarrow [Al(EtOH)_5(DPPH-H)]^{3+}$	−0.52	−0.84
D10. $[Al(EtOH)_5(DPPH\cdot)]^{3+} + e^- \rightarrow [Al(EtOH)_4(EtO^-)(DPPH-H)]^{2+}$	1.65	1.38
D11. $[Al(EtOH)_4(EtO^-)(DPPH\cdot)]^{2+} + e^- \rightarrow [Al(EtOH)_4(EtO^-)(DPPH^-)]^{+}$	0.98	0.71
D12. $[Al(EtOH)_4(EtO^-)(DPPH\cdot)]^{2+} + H^+ + e^- \rightarrow [Al(EtOH)_4(EtO^-)(DPPH-H)]^{+2}$	0.75	0.45
D13. $[Al(EtOH)_4(EtO^-)(DPPH\cdot)]^{2+} + e^- \rightarrow [Al(EtOH)_3(EtO^-)_2(DPPH-H)]^{+}$	1.01	0.74
Exp. Ref.	+0.70	0.47

[a] In the case of D1, D2, D5a, D5b, D8 and D10 the reactions are classified as electron transfer process and therefore, a value of 0.27 eV have been considered as the theoretical reference of the reduction potential without aluminum. In the case of D3, D4, D6, D7 and D9, since the reactions are of proton-coupled electron-transfer type, we consider +0.32 eV as the reference in absence of aluminum.

present at the experimental pH value according the results of Table 3. In other words, their formation has an extra energetic cost and these complexes are less likely to exist.

All the potential reactions D5a, D5b, and D7 evolve from a divalent Al^{3+}-complex to a monovalent one. The significance of the charge reduction is clearly demonstrated if we compare D6 and D7 reduction reactions. Both reactions imply the reduction of DPPH· with its further protonation and coordination to Al^{3+}. While in reaction D7 the protonation takes place at the expense of the deprotonation of an ethanol ligand already coordinated to Al^{3+} (Fig. 4c), reducing the final charge of the Al^{3+}-complex to +1, in reaction D6 the DPPH· protonation is assisted by an extra proton of the media that makes the final charge of the Al^{3+}-complex to be +2. This small difference between the two reactions completely changes their thermochemistry, evolving from a spontaneous reaction (D7) with a E_{red} that perfectly matches with experimental data to a non-spontaneous reaction (D6) with a E_{red} that is absolutely in disagreement with experimental results. The difference between reactions D5a and D5b lies on the relative coordination of $DPPH^-$ and the deprotonated ethanol group (in cis for the D5a reaction and in trans for D5b) (see Fig. 4) while they describe the same chemical reaction.

Specifically, the reduction reaction that presents the E_{red} with highest agreement with the experimental value is D7. This reaction accounts for

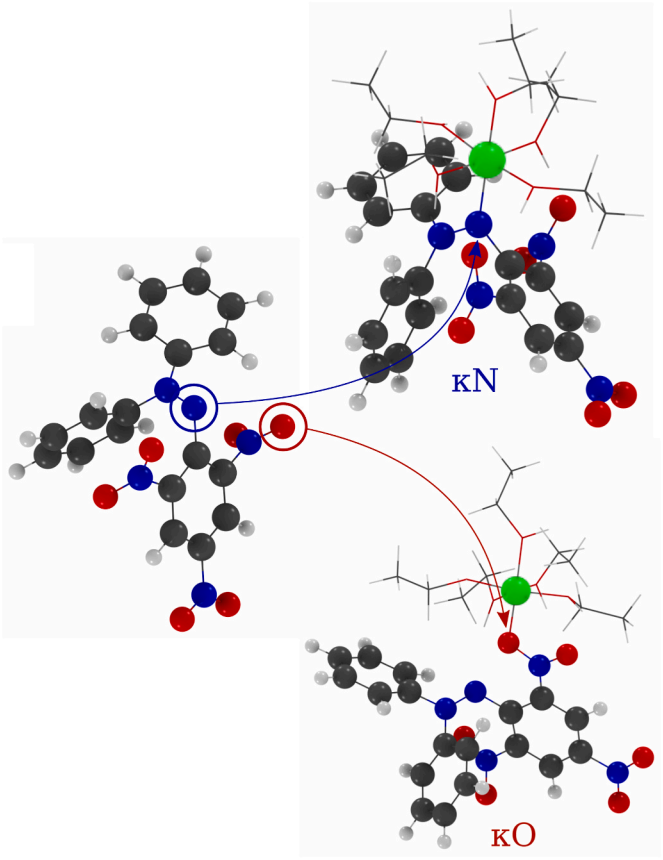

Fig. 3. Coordination modes of DPPH·. In blue, through the nitrogen atom of the diazane moiety. In red, through one oxygen atom of an orto nitro group of the picryl unit. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the reduction of DPPH· coupled to its coordination to Al^{3+} and an intramolecular protonation assisted by an ethanol ligand. Once again, as we saw for hydroquinones and for the redox reactions without the presence of Al^{3+}, the global redox process is better described as a PCET.

If we compare calculated reduction potentials of DPPH· considering the presence of Al^{3+} (Table 6) with the ones without Al^{3+} presence (d in Table 2), we can see that the presence of aluminum increases the E_{red} values ($\Delta E_{red}^{\varnothing \rightarrow Al}$), leading to a more exergonic process and stabilizing the reduced DPPH-H species.

4. Overall redox reaction and biological implications

Our theoretical results for the reduction/oxidation potentials in the presence/absence of aluminum and upon methylation of hydroquinones reproduce the experimental trends, validating the methodology used. Based on our results, the DPPH· scavenging reaction of hydroquinones, namely,

$$DPPH\cdot + QH_2 \xrightarrow{Al^{3+}} DPPH\text{–}H + QH\cdot \quad (3)$$

would proceed via a proton coupled electron transfer process, and the corresponding free energy changes (ΔG_{pcet}) can be found in Table 7 for the different cases: absence/presence of aluminum and methylation.

It is clear from the data of Table 7 that aluminum acts as a promoter of this scavenging reaction, passing from an endergonic process in the absence of aluminum (+0.16/+0.08 V for exp/theo values) to an exergonic spontaneous reaction when aluminum is present (-0.15/-0.05 V). The methylation of hydroquinones also promotes the scavenging reaction. We observe synergy between methylation and the presence of

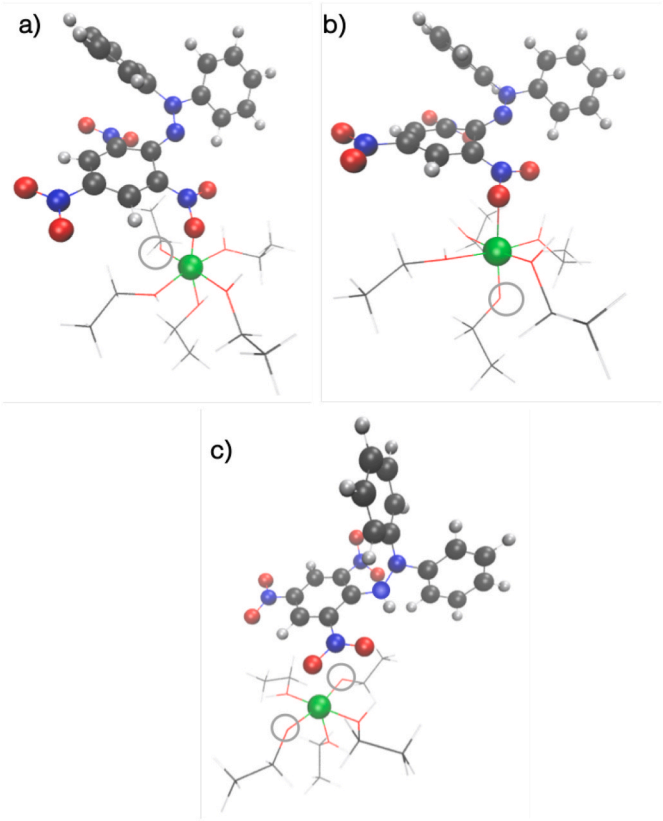

Fig. 4. Al^{3+} complexes formed as products of the DPPH· reduction. a) $Al(EtOH)_4(EtO^-)(DPPH^-)]^+$ product from reaction D5a, with $DPPH^-$ and the deprotonated ethanol group in cis. b) $[Al(EtOH)_4(EtO^-)(DPPH^-)]^+$ product from reaction D5b with $DPPH^-$ and the deprotonated ethanol group in trans. c) $[Al(EtOH)_3(EtO^-)_2(DPPH\text{-}H)]^+$ product from reaction D7. Deprotonated ethanol ligands are pointed with gray circles.

Table 7
Free energy change (ΔG_{pcet}) of the proton coupled electron transfer processes (in V). ΔG is calculated as $\Delta G_{pcet} = -F(E_{ox} + E_{red})$. E_{ox} of hydroquinones and E_{red} of DPPH·.

Redox reactions	No Aluminum		Aluminum	
	Exp.	Theo	Exp.	Theo
$QH_2 + DPPH\cdot \rightarrow QH\cdot + DPPH - H$	+0.16	+0.08	−0.15	−0.05
$MeQH_2 + DPPH\cdot \rightarrow MeQH\cdot + DPPH - H$	−0.04	+0.04	−0.42	−0.28

aluminum, obtaining the highest exergonicity with a value of −0.42/-0.28 V for the reaction with methylated hydroquinone in the presence of aluminum. In summary, aluminum, albeit a non-redox metal, can enhance the electron transfer process, and it does so by strongly stabilizing the reduced form of DPPH·.

Our theoretical results allow us to get insights into the origins of this stabilization. On the one hand, there is an electrostatic stabilization of the highly charged Al^{3+} on the increase of negative charge with the reduced $DPPH^-$ species. On the other hand, a proton transfer from one of the ethanols bound to aluminum further stabilizes the formal $DPPH^-$, forming a DPPH−H species. This internal proton transfer increases the local negative charge around the aluminum atom in the first coordination shell, because the unprotonated ethanol is directly bound to the metal in contrast to the diazine nitrogen. In addition, this proton transfer stabilizes the excess of negative charge at the diazine nitrogen upon electron transfer. Together, all these effects make the aluminum promotion of the DPPH·'s reduction higher than the decrease of electron donor ability of hydroquinones. Therefore, the net outcome is a promotion of the overall redox reaction. Thus, the present work demonstrates how a strong Lewis acid such as aluminum can alter the thermodynamics of a paradigmatic test-case redox reaction. This finding has high relevance in a biological context since one would expect that the presence of aluminum has consequences for the proper and necessary balance of free radicals in a biological medium.

Free radicals are species that have an independent existence, for however brief a period, and contain at least one unpaired electron. The production of free radicals is inherent to normal cell behavior. Many redox processes produce and consume them. Superoxide ($O_2^{\cdot-}$) is the most typical oxygen-containing species enclosing a free radical. It is formed as a side product of the energy production process in the mitochondrion. $O_2^{\cdot-}$, peroxides and hydroxyl radicals (OH·) are included under the general heading of reactive oxygen species (ROS). The unbalance of free radicals leads to the so-called oxidative stress, and it is at the origin of multiple diseases like cancer, diabetes, Alzheimer's, or Parkinson's diseases [49,50]. The human brain is a highly aerobic organ with high oxygen consumption, with a high energy requirement of neurons driven by mitochondrial oxidative phosphorylation. Part of this oxygen is converted into ROS, which in a healthy individual are effectively detoxified by several antioxidants, like enzymes (glutathione peroxidase and catalase for hydrogen peroxide [51], and superoxide dismutase for superoxide [52]) or low molecular mass antioxidants [53,54]. These antioxidants can perform a radical-scavenging process with ROS, receiving/donating an electron from/to a radical, forming stable byproducts. For instance, in lipid peroxidation within the mitochondrial membranes, the reduced form of the coenzyme Q_{10} (ubiquinol, UQH_2) acts as the main chain-breaking antioxidant that decreases the damage of this process [55–57]. The active center of the Q_{10} antioxidant is precisely formed by hydroquinone (QH_2).

However, ROS increases markedly and uncontrolled in an aged brain or under different circumstances with mitochondrial dysfunctions [58]. In this context, the presence and accumulation of exometals can induce or enhance oxidative stress in the cell. The co-localization of aluminum and neurofibrillary tangles in familial Alzheimer's disease and aluminum and iron in nuclei of nerve cells in the brains of patients with Alzheimer's disease have been recently unambiguously established [58,59]. The presence of aluminum, in addition to the well-known implication of oxidative stress in neurodegeneration, suggests that this metal can promote oxidative damage to DNA or inhibit the repair of oxidatively damaged DNA. In previous work [8,10], we demonstrated how aluminum could thermodynamically stabilize a superoxide anion, promoting Fenton reaction and the generation of radical species. In the present work, we underline the effect aluminum could have in promoting a radical scavenging reaction. Both phenomena are of different nature, in that in the former, there is a promotion of oxidative activity, whereas, in the latter, there is a protective effect. However, both cases have in common the ability of aluminum to alter the thermodynamics of key redox processes in biological systems. Indeed, long-term exposure to $AlCl_3$ even at a low dose promotes oxidative stress [60,61], although high aluminum concentrations tend to decrease oxidative stress in zebrafish [62]. In any case, aluminum is affecting the concentration of radical species. This behavior is due to the physicochemical properties of Al^{+3}, a strong Lewis acid with a substantial stabilization ability of negatively charged ions. This property allows aluminum to alter the thermodynamics of biological essential redox processes in different and subtle ways.

5. Conclusions

We have presented an accurate theoretical evaluation of reduction/oxidation potentials in the presence/absence of aluminum for a test case redox reaction between DPPH· and hydroquinone. The relevant species' reduction and oxidation potentials have been previously determined experimentally in the absence and presence of aluminum, allowing comparison between experimental and theoretical data provided in this

work. To the best of our knowledge, this is the first time that such a comparison has been made in the context of aluminum biochemistry. The results given here support the experimental predictions that aluminum alters the thermodynamics of the scavenging reaction, being able to shift the process from an endergonic situation to a spontaneous exergonic one.

At the origin of this effect, there is the ability of Al^{+3} to stabilize the reduced form of DPPH·, not only by stabilizing the negatively charged $DPPH^{-}$ but also by promoting proton transfer from the first shell ethanol molecules to the nitrogen of diazine. This proton transfer is favorable due to the significant lowering of the pKa of ligands bound to aluminum [9]. All these properties stem from the fact that aluminum is a very strong Lewis acid, which can differentially stabilize charged species formed in redox reactions without the need to receive/release electrons directly.

Consequently, we can conclude that the presence of aluminum in biological media can not be considered an inert factor since it can affect the thermodynamic equilibrium of processes in which the production/scavenging of radicals takes part. The alteration of the proper balance of radicals in biological media due to the presence of aluminum could be behind some of the most important toxic effects of aluminum in biological media, as early hypothesized in the literature [14,16] but often underestimated due to the non-redox nature of this exogenous metal, and the long-held prejudice to consider aluminum as an intrinsically inert agent in biological medium.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.freeradbiomed.2021.12.308.

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