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ORIGINAL PAPER

# PM6 study of free radical scavenging mechanisms of flavonoids: why does O–H bond dissociation enthalpy effectively represent free radical scavenging activity?

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**Abstract** It is well known that the bond dissociation enthalpy (BDE) of the O–H group is related to the hydrogen atom transfer (HAT) mechanism of free radical scavenging that is preferred in gas-phase and non-polar solvents. The present work shows that the BDE may also be related to radical scavenging processes taking place in polar solvents, i.e., single electron transfer followed by proton transfer (SET-PT) and sequential proton loss electron transfer (SPLET). This is so because the total energy requirements related to the SET-PT [sum of the ionization potential (IP) and proton dissociation enthalpy (PDE)] and the SPLET [sum of the proton affinity (PA) and electron transfer enthalpy (ETE)] are perfectly correlated with the BDE. This could explain why the published data for polyphenolic antioxidant activity measured by various assays are better correlated with the BDE than with other reaction enthalpies involved in radical scavenging mechanisms, i.e., the IP, PDE, PA and ETE. The BDE is fairly well able to rank flavonoids as antioxidants in any medium, but to conclude which radical scavenging mechanism represents the most probable reaction pathway from the thermodynamic point of view, the IP and PA (ETE) should also be considered. This is exemplified in the case of the radical scavenging activity of 25 flavonoids.

**Keywords** Flavonoids · Radical scavenging · Bond dissociation enthalpy · Hydrogen atom transfer · Single electron transfer followed by proton transfer · Sequential proton loss electron transfer

**Abbreviations**

| | |
|---|---|
| HAT | Hydrogen atom transfer |
| BDE | Bond dissociation enthalpy |
| SET-PT | Single electron transfer followed by proton transfer |
| IP | Ionization potential |
| PDE | Proton dissociation enthalpy |
| SPLET | Sequential proton loss electron transfer |
| PA | Proton affinity |
| ETE | Electron transfer enthalpy |

## Introduction

Oxidative stress induced by free radicals, such as the lipid peroxyl radical LOO• and the hydroxyl radical •OH, can cause damage to cellular proteins, membrane lipids and nucleic acids. This process has been implicated in the pathogenesis of various diseases, including coronary heart disease and some forms of cancer [1]. Flavonoids are natural polyphenolic multifunctional antioxidants capable of

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combating free radicals by direct scavenging, chelating metal ions, inhibiting prooxidant enzymes and activating antioxidant and detoxifying enzymes [2, 3]. Due to their protective effects, flavonoids are recognized as potential drug candidates to be used in the treatment of diseases such as atherosclerosis, cardiovascular and coronary heart diseases, cancer, neurodegenerative diseases such as Parkinson's and Alzheimer's diseases, and other age-related diseases [4, 5].

The scavenging of free radicals seems to play a notable role in the antioxidant activity of flavonoid compounds. The antiradical properties of flavonoids (FlO–H) are related to their ability to transfer their phenolic H-atom to a free radical (e.g., alkoxyl radical, RO•). The formal H-atom abstraction from flavonoids described by:

$$\mathrm{Fl-OH + RO^{\bullet} \rightarrow Fl-O^{\bullet} + ROH} \quad (1)$$

is known to involve complex processes. It has been recognized that this reaction proceeds via at least three different mechanisms [6–9]: single-step hydrogen atom transfer (HAT), single electron transfer followed by proton transfer (SET-PT) and sequential proton loss electron transfer (SPLET). These mechanisms may co-exist, and they depend on solvent properties and radical characteristics. The net result from all mechanisms is the same, i.e., as given in reaction (1).

In the HAT mechanism, the phenolic hydrogen atom is transferred to the free radical. The product of this reaction is a flavonoid phenoxyl radical (Fl–O•). To be effective, the Fl–O• must be a relatively stable free radical so that it reacts slowly with a substrate but rapidly with RO•. The HAT mechanism can be characterized by the homolytic bond dissociation enthalpy (BDE) of the OH group. The O–H BDE can be calculated by the following equation:

$$\mathrm{BDE = H(Fl-O^{\bullet}) + H(H) - H(Fl-OH)} \quad (2)$$

*H*(Fl–O•) is the enthalpy of the flavonoid phenoxyl radical generated after H• abstraction, *H*(H) is the enthalpy of the hydrogen atom, and *H*(Fl–OH) is the enthalpy of the parent flavonoid molecule. A lower BDE value, usually attributed to a greater ability to donate a hydrogen atom from the hydroxyl group, results in an easier free radical scavenging reaction. HAT is favored for radicals with a high H-atom affinity and is preferred in non-polar solvents because it does not involve charge separation [10]. Wright et al. [6] have suggested that BDE is an excellent primary descriptor of the antioxidant activity.

In the SET-PT mechanism, the first step is the transfer of an electron by which the flavonoid radical cation Fl–$OH^{\bullet+}$ is formed.

$$\mathrm{Fl-OH \rightarrow Fl-OH^{\bullet+} + e^{-}} \quad (3)$$

This step can be characterized by the ionization potential (IP). IP is a global property of the molecule. It can be calculated as follows:

$$\mathrm{IP = H(Fl-OH^{\bullet+}) + H(e^{-}) - H(Fl-OH)} \quad (4)$$

*H*(Fl–$OH^{\bullet+}$) is the enthalpy of the flavonoid radical cation generated after electron abstraction, and *H*($e^{-}$) is the enthalpy of the electron.

The second step is the deprotonation of Fl–$OH^{\bullet+}$:

$$\mathrm{Fl-OH^{\bullet+} \rightarrow Fl-O^{\bullet} + H^{+}} \quad (5)$$

It can be described by the O–H proton dissociation enthalpy (PDE) which can be calculated by the following equation:

$$\mathrm{PDE = H(Fl-O^{\bullet}) + H(H^{+}) - H(Fl-OH^{\bullet+})} \quad (6)$$

*H*($H^{+}$) is the enthalpy of the proton. The net result of the SET-PT mechanism is the same as in the HAT mechanism—the formation of a corresponding flavonoid radical.

Deprotonation of the flavonoid molecule, which results in the formation of a phenoxide anion Fl–$O^{-}$, is the first step in the SPLET mechanism [8, 11]:

$$\mathrm{Fl-OH \rightarrow Fl-O^{-} + H^{+}} \quad (7)$$

This step corresponds to the proton affinity (PA) of the phenoxide anion Fl–$O^{-}$. PA can be calculated by the following equation:

$$\mathrm{PA} = H(\mathrm{Fl-O^{-}}) + H(\mathrm{H^{+}}) - H(\mathrm{Fl-OH}) \quad (8)$$

*H*(Fl–$O^{-}$) is the enthalpy of the flavonoid anion generated after proton abstraction. In the second step, electron transfer from Fl–$O^{-}$ takes place:

$$\mathrm{Fl-O^{-} \rightarrow Fl-O^{\bullet} + e^{-}} \quad (9)$$

This step is related to the electron transfer enthalpy (ETE). The ETE can be determined by the following equation:

$$\mathrm{ETE} = H(\mathrm{Fl-O^{\bullet}}) + H(\mathrm{e^{-}}) - H(\mathrm{Fl-O^{-}}) \quad (10)$$

The net result of SPLET is again the same as in HAT and SET-PT—the formation of the corresponding radical. The SET-PT and SPLET mechanisms are favored in polar media because of the charge separation. They are preferred for radicals with a high electron affinity [10].

In our previous reports we demonstrated that the fast semiempirical PM6 method is fairly well able to reproduce DFT results regarding reaction enthalpies involved in free radical scavenging mechanisms [12–14]. The aim of the present work was to ascertain correlations between the experimental radical scavenging activity (RSA) of 25 flavonoids [15] and the calculated reaction enthalpies (BDE, IP, PDE, PA and ETE) related to three free radical scavenging

mechanisms (HAT, SET-PT and SPLET). On the basis of the results obtained, conclusions can be drawn regarding the potency of the BDE as a major physico-chemical parameter that correlates with the free radical scavenging activity of flavonoids [16, 17]. It should be noted that thermodynamic parameters may be important factors governing the radical scavenging reactions of flavonoids, while the more complete insight would certainly require kinetic analysis.

## Computational details

All calculations were performed using the MOPAC2009™ program package [18]. The geometries of 25 flavonoids and the corresponding radicals, anions and radical cations were optimized using the PM6 method. The eigenvector following (EF) optimization procedure was carried out with a final gradient norm under 0.01 kcal$mol^{-1}$ $Å^{-1}$. The solvent contribution to the enthalpies of formation was computed by employing the COSMO (conductor-like screening model) calculations implemented in MOPAC2009™. This approach was used for all structures. The hydration enthalpies of the hydrogen atom (H•), proton ($H^+$) and electron ($e^-$) were taken from the literature [9, 19].

## Results and discussion

The results of calculations (in water) of reaction enthalpies for 25 flavonoids, related to the individual steps of three free radical scavenging mechanisms are presented in Table 1. The corresponding results in the gas-phase are presented in Table 2. The radical scavenging potency of flavonoids is related primarily to the presence of OH groups at specific positions on the flavonoid core. Depending on the reaction medium, homolytic or heterolytic O–H bond cleavage could take place at active site on the flavonoid core. Comparisons of the calculated reaction enthalpies, i.e., BDE, IP, PDE, PA and ETE, enable prediction of the thermodynamically preferred reaction pathway and the active site for free radical inactivation. Among the descriptors related to the antiradical activity, the BDE of the O–H group is a theoretical parameter that can be used successfully to measure the H-atom donating ability of various antioxidants [20–23]. The minimal value of the BDE of O–H bonds (BDEmin) indicates which O–H group on the flavonoid core possesses the most abstractable hydrogen, that is, which O–H group is targeted for radical attack. Such a feature is related primarily to the catechol moiety in the B ring and the 3-OH group of the C ring [24]. The BDEmin could serve as a theoretical measure for ranking flavonoids as antioxidants because most active flavonoids possess lower values of the BDE.

### Free radical scavenging activity of quercetin

In this section, we present and discuss results obtained for quercetin (Fig. 1) as an illustrative example of the 25 studied flavonoids. As a potent antioxidant, quercetin is studied widely using both experimental and theoretical approaches [25–29].

The preferred mechanism of the antiradical activity of flavonoids can be estimated from values of the BDE (HAT mechanism), IP (first step of the SET-PT mechanism) and PA (first step of the SPLET mechanism) [9, 19]. The lowest value indicates which mechanism is thermodynamically the most probable process. For quercetin in water, the reaction enthalpies (in kJ$mol^{-1}$) are shown in Fig. 1. The PA values (14–51 kJ$mol^{-1}$) are lower than the IP (361 kJ$mol^{-1}$) and BDE (298–383 kJ$mol^{-1}$) values, which indicates that SPLET is the preferred mechanism in water. It should be noted that the ETE values (260–368 kJ$mol^{-1}$) are lower than the corresponding BDE values, as well as the IP value, which further indicates that in water, the SPLET mechanism is thermodynamically favorable.

The preferred site of antioxidant action may be estimated from the minimal sum of enthalpies involved in a particular free radical scavenging mechanism. For the HAT mechanism, it is simply the minimal value of the BDE (BDEmin) that accounts for the one-step H-atom transfer. In the case of the SET-PT mechanism, this sum includes the IP and PDE [min(IP + PDE)], and in the SPLET mechanism, the sum includes the PA and ETE [min(PA + ETE)]. The minimal energy requirement for homolytic bond cleavage, i.e., BDEmin is related to the 4′-OH group of quercetin (Fig. 1). Therefore, if the HAT mechanism occurs, the thermodynamically preferred site for radical inactivation is the 4′-OH group. An important influence on the free radical scavenging activity of phenolics is the pH of the surrounding media [29–31]. In polar solvents that support ionization, the antioxidant action of flavonoids occurs primarily by the SPLET mechanism. The kinetic measurements show that scavenging reactions in polar solvents are affected by the acidity of flavonoid compounds, i.e., by the amount of accessible phenoxide anions [8]. At a physiological pH of 7.4, one of OH groups of quercetin loses a proton [29]. The min(PA + ETE) is characteristic of the 4′-OH group of quercetin (Fig. 1). According to the lowest PA value, the 7-OH group of quercetin is the preferred site for heterolytic bond cleavage and for entering the SPLET mechanism. However, charge redistribution and resonance stabilization [29] result in formation of the most stable 4′-O• radical—the one formed at site with minimal energy requirements. In the case of the SET-PT mechanism, the min(IP + PDE) value is also associated with this OH group. Therefore, if the SPLET (SET-PT) mechanism occurs, the 4′-O• phenoxyl radical of quercetin will be formed.

**Table 1** Conductor-like screening model (COSMO) PM6 reaction enthalpies ($kJ\,mol^{-1}$) in water for 25 neutral molecules of flavonoids. Radical scavenging activity (RSA) denotes experimental antiradical activity (percentage of 1,1-diphenyl-2-picrylhydrazyl (DPPH) decoloration [15]). *BDE* bond dissociation enthalpy, *IP* ionization potential, *PDE* proton dissociation enthalpy, *PA* proton affinity, *ETE* electron transfer enthalpy

| | Flavonoid | Active site | BDE | IP | PDE | IP + PDE | PA | ETE | PA + ETE | RSA |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Morin | 3-OH | 317.89 | 381.16 | −63.92 | 317.24 | 11.79 | 305.44 | 317.23 | 96.5 |
| 2 | Taxifolin | 4′-OH | 310.54 | 365.80 | −55.91 | 309.89 | 55.90 | 253.99 | 309.89 | 94.8 |
| 3 | Kaempferol | 3-OH | 308.91 | 360.86 | −52.61 | 308.25 | 44.29 | 263.96 | 308.25 | 93.5 |
| 4 | Fustin | 4′-OH | 308.02 | 365.55 | −58.19 | 307.36 | 40.53 | 266.83 | 307.36 | 91.9 |
| 5 | Galangin | 3-OH | 308.11 | 377.20 | −69.74 | 307.46 | 39.00 | 268.46 | 307.46 | 91.8 |
| 6 | Rutin | 3′-OH | 312.74 | 377.01 | −64.93 | 312.08 | 45.39 | 266.69 | 312.08 | 90.9 |
| 7 | Quercetin | 4′-OH | 298.40 | 361.13 | −63.38 | 297.75 | 30.89 | 266.86 | 297.75 | 89.8 |
| 8 | Luteolin 7-glucoside | 4′-OH | 313.10 | 377.88 | −65.44 | 312.44 | 22.59 | 289.86 | 312.45 | 87.6 |
| 9 | Quercetin 3-glucoside-7-rhamnoside | 4′-OH | 310.37 | 381.13 | −71.42 | 309.71 | 25.91 | 283.80 | 309.71 | 86.8 |
| 10 | Laricytrin | 4′-OH | 288.14 | 357.11 | −69.62 | 287.49 | 42.55 | 244.93 | 287.48 | 84.6 |
| 11 | Laricytrin 3′-glucoside | 4′-OH | 286.40 | 357.54 | −71.80 | 285.74 | 35.51 | 250.23 | 285.74 | 83.8 |
| 12 | Robinetin | 4′-OH | 289.96 | 358.82 | −69.51 | 289.31 | 41.84 | 247.47 | 289.31 | 82.3 |
| 13 | Fisetin | 4′-OH | 297.24 | 358.53 | −61.94 | 296.59 | 32.75 | 263.84 | 296.59 | 79.0 |
| 14 | Myricetin | 4′-OH | 289.82 | 360.85 | −71.69 | 289.16 | 40.08 | 249.09 | 289.17 | 72.8 |
| 15 | Kaempferol 3,7-dirhamnoside | 4′-OH | 331.00 | 397.39 | −67.04 | 330.35 | 38.29 | 292.05 | 330.34 | 70.6 |
| 16 | 3-OH Flavone | 3-OH | 308.91 | 370.38 | −62.13 | 308.25 | 48.17 | 260.09 | 308.26 | 66.0 |
| 17 | Apigenin 7-glucoside | 4′-OH | 343.43 | 404.84 | −62.07 | 342.77 | 32.28 | 310.49 | 342.77 | 34.8 |
| 18 | Hesperetin | 3′-OH | 308.59 | 360.07 | −52.14 | 307.93 | 55.42 | 252.52 | 307.94 | 30.0 |
| 19 | Vitexin | 4′-OH | 342.60 | 404.12 | −62.18 | 341.94 | 36.64 | 305.30 | 341.94 | 21.0 |
| 20 | Naringenin | 4′-OH | 333.16 | 404.21 | −71.71 | 332.50 | 53.77 | 278.74 | 332.51 | 6.3 |
| 21 | Naringin | 4′-OH | 333.46 | 390.07 | −57.27 | 332.80 | 50.75 | 282.05 | 332.80 | 4.7 |
| 22 | 7-OH Flavone | 7-OH | 362.43 | 447.01 | −85.23 | 361.78 | 23.02 | 338.75 | 361.77 | 2.8 |
| 23 | Chrysin | 5-OH | 380.06 | 428.51 | −49.10 | 379.41 | 32.97 | 346.44 | 379.41 | 1.1 |
| 24 | Apigenin | 4′-OH | 341.46 | 423.45 | −82.64 | 340.81 | 34.20 | 306.61 | 340.81 | 0.7 |
| 25 | 5-OH Flavone | 5-OH | 356.81 | 400.35 | −44.19 | 356.16 | 39.40 | 316.75 | 356.15 | 0.6 |
| | | | $r$=−0.802[a] $s$=23.01[b] | $r$=−0.777 $s$=24.23 | $r$=0.126 $s$=36.99 | $r$=−0.802 $s$=23.02 | $r$=−0.123 $s$=38.21 | $r$=−0.648 $s$=29.33 | $r$=−0.802 $s$=23.01 | |

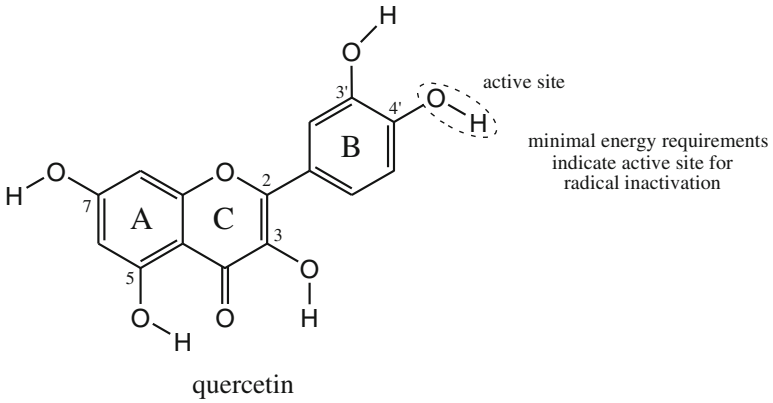
[a] Correlation coefficient

[b] Standard error of estimate

**Table 2** PM6 reaction enthalpies ($kJmol^{-1}$) in the gas-phase for 25 neutral molecules of flavonoids

| | Flavonoid | Active site | BDE | IP | PDE | IP + PDE | PA | ETE | PA + ETE | RSA |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Morin | 3-OH | 322.34 | 761.16 | 882.52 | 1643.68 | 1294.43 | 349.25 | 1643.68 | 96.5 |
| 2 | Taxifolin | 3′-OH | 309.09 | 779.70 | 850.74 | 1630.44 | 1342.03 | 288.41 | 1630.44 | 94.8 |
| 3 | Kaempferol | 3-OH | 319.28 | 749.00 | 891.63 | 1640.63 | 1338.52 | 302.11 | 1640.63 | 93.5 |
| 4 | Fustin | 4′-OH | 304.99 | 774.11 | 852.23 | 1626.34 | 1334.66 | 291.68 | 1626.34 | 91.9 |
| 5 | Galangin | 3-OH | 318.62 | 768.65 | 871.32 | 1639.97 | 1335.16 | 304.80 | 1639.96 | 91.8 |
| 6 | Rutin | 4′-OH | 311.39 | 748.88 | 883.86 | 1632.74 | 1295.16 | 337.59 | 1632.75 | 90.9 |
| 7 | Quercetin | 4′-OH | 295.69 | 759.27 | 857.77 | 1617.04 | 1297.00 | 320.04 | 1617.04 | 89.8 |
| 8 | Luteolin 7-glucoside | 4′-OH | 310.27 | 817.77 | 813.85 | 1631.62 | 1270.29 | 361.33 | 1631.62 | 87.6 |
| 9 | Quercetin 3-glucoside-7-rhamnoside | 4′-OH | 304.28 | 755.11 | 870.51 | 1625.62 | 1268.67 | 356.96 | 1625.63 | 86.8 |
| 10 | Laricytrin | 4′-OH | 296.61 | 747.65 | 870.31 | 1617.96 | 1327.74 | 290.21 | 1617.95 | 84.6 |
| 11 | Laricytrin 3′-glucoside | 4′-OH | 298.19 | 725.16 | 894.37 | 1619.53 | 1284.02 | 335.51 | 1619.53 | 83.8 |
| 12 | Robinetin | 4′-OH | 299.80 | 751.99 | 869.15 | 1621.14 | 1339.34 | 281.81 | 1621.15 | 82.3 |
| 13 | Fisetin | 4′-OH | 294.11 | 748.39 | 867.06 | 1615.45 | 1313.30 | 302.15 | 1615.45 | 79.0 |
| 14 | Myricetin | 4′-OH | 300.38 | 761.36 | 860.36 | 1621.72 | 1322.34 | 299.38 | 1621.72 | 72.8 |
| 15 | Kaempferol 3,7-dirhamnoside | 4′-OH | 334.49 | 725.00 | 930.83 | 1655.83 | 1292.18 | 363.66 | 1655.84 | 70.6 |
| 16 | 3-OH Flavone | 3-OH | 318.34 | 758.77 | 880.91 | 1639.68 | 1360.01 | 279.66 | 1639.67 | 66.0 |
| 17 | Apigenin 7-glucoside | 4′-OH | 343.87 | 800.25 | 864.97 | 1665.22 | 1287.70 | 377.51 | 1665.21 | 34.8 |
| 18 | Hesperetin | 3′-OH | 324.42 | 776.63 | 869.14 | 1645.77 | 1380.80 | 264.96 | 1645.76 | 30.0 |
| 19 | Vitexin | 4′-OH | 341.83 | 803.03 | 860.14 | 1663.17 | 1300.57 | 362.60 | 1663.17 | 21.0 |
| 20 | Naringenin | 4′-OH | 338.07 | 827.22 | 832.20 | 1659.42 | 1344.36 | 315.05 | 1659.41 | 6.3 |
| 21 | Naringin | 4′-OH | 337.91 | 822.23 | 837.03 | 1659.26 | 1317.19 | 342.08 | 1659.27 | 4.7 |
| 22 | 7-OH Flavone | 7-OH | 357.77 | 835.74 | 843.38 | 1679.12 | 1322.11 | 357.01 | 1679.12 | 2.8 |
| 23 | Chrysin | 7-OH | 386.20 | 798.56 | 908.99 | 1707.55 | 1318.29 | 389.26 | 1707.55 | 1.1 |
| 24 | Apigenin | 4′-OH | 345.25 | 843.21 | 823.39 | 1666.60 | 1300.14 | 366.46 | 1666.60 | 0.7 |
| 25 | 5-OH Flavone | 5-OH | 394.63 | 777.49 | 938.49 | 1715.98 | 1382.87 | 333.10 | 1715.97 | 0.6 |
| | | | $r$=−0.830<br>$s$=21.49 | $r$=−0.729<br>$s$=26.36 | $r$=0.108<br>$s$=38.28 | $r$=−0.830<br>$s$=21.49 | $r$=−0.270<br>$s$=37.07 | $r$=−0.401<br>$s$=35.27 | $r$=−0.830<br>$s$=21.49 | |

**Fig. 1** Reaction enthalpies (in $kJmol^{-1}$) for quercetin calculated by the COSMO PM6 method (in water) involved in the hydrogen atom transfer (HAT), single electron transfer followed by proton transfer (SET-PT) and sequential proton loss electron transfer (SPLET) mechanisms




quercetin

| | HAT | | SET-PT | | | SPLET |
|---|---|---|---|---|---|---|
| | BDE | IP | PDE | IP + PDE | PA | ETE | PA + ETE |
| | | 361 | | | | | |
| 3-OH | 305 | | -57 | 304 | 35 | 269 | 304 |
| 5-OH | 373 | | 11 | 372 | 22 | 350 | 372 |
| 7-OH | 383 | | 21 | 382 | 14 | 368 | 382 |
| 3'-OH | 311 | | -51 | 310 | 51 | 260 | 311 |
| 4'-OH | 298 | | -63 | 298 | 31 | 267 | 298 |

active site for radical inactivation — BDEmin — min(IP + PDE) — min(PA + ETE)

From the previous analysis, we can observe that the thermodynamically preferred mechanism of free radical scavenging by quercetin in water is the SPLET mechanism because of the lowest PA (and ETE) values. As shown in Fig. 1, the minimal energy requirements, i.e., BDEmin, min (IP + PDE) and min(PA + ETE), are associated with the 4′-OH group of quercetin, and this group is the preferred site for radical inactivation by all three mechanisms. In agreement with this, the performed PM6 calculations indicate that the 4′-O• phenoxyl radical of quercetin is the lowest energy radical with spin densities delocalized over the cinnamoyl part (C and B rings) of the molecule, which contributes to the stability of the radical (Fig. 2). Published DFT results also indicate the 4′-O• phenoxyl radical of quercetin as the most stable [24–26, 29, 32]. In the gas-phase, the HAT mechanism is dominant because the BDEs of OH groups of quercetin are significantly lower than the IP and PAs (Table 2, quercetin).

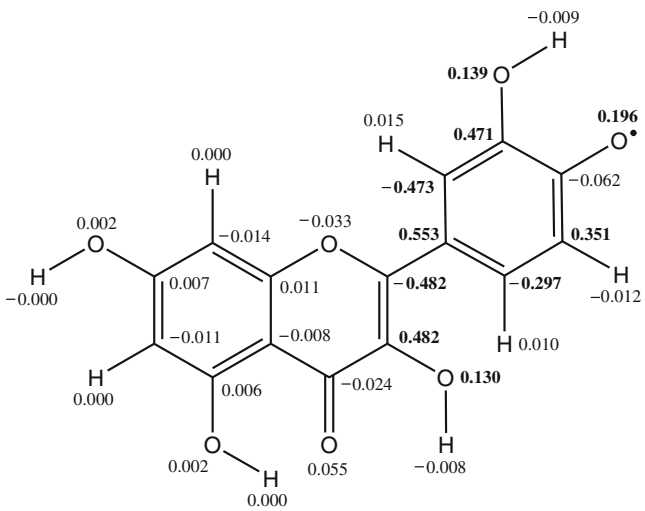



**Fig. 2** Spin densities of the 4′-O• phenoxyl radical of quercetin (in water)

## BDE as a general descriptor of free radical scavenging potency

The BDE of the O–H group has been recognized as a useful descriptor in structure-activity analyses of antioxidants such as flavonoids [33]. A number of DFT calculations of the O–H BDE of flavonoids have been reported [34–38]. The O–H BDE calculations explain that flavonoids with OH groups in the ortho-positions can exhibit high free radical scavenging activity [39]. In different media the same compound may react with the same free radical by different mechanisms depending on free radical properties, pH value and polarity of the medium [10, 40, 41]. Despite that, numerous reports on the antiradical activity of polyphenols usually find, albeit

not yet fully recognized, superior correlation with O–H BDE. Anouar et al. [16] reported that BDE is able to partly describe energetics related to HAT, SPLET and SET-PT mechanisms. In this section, we address this fact in terms of the energy requirements for the reaction pathways involved in the free radical scavenging mechanisms.

In the previous section, we illustrated that the minimal energy requirements for the HAT (BDEmin), SET-PT [min (IP + PDE)] and SPLET [min(PA + ETE)] are associated with the same OH group of flavonoid compounds and that the final product of all free radical scavenging mechanisms is the same: the thermodynamically most stable flavonoid phenoxyl radical. Here, we focused on the intercorrelation between energetics related to the HAT, SET-PT and SPLET mechanisms.

The total energy requirement for the SET-PT mechanism (Eq. 11 in Fig. 3) is the sum of the IP (Eq. 4) and PDE (Eq. 6). As in Eq. 2, in which the BDE represents the energy change related to HAT, the energy requirement for SET-PT also includes the difference in the enthalpies of the parent molecule and the corresponding phenoxyl radical. This difference is corrected by the hydrogen atom enthalpy in Eq. 2 and by the sum of the proton and electron enthalpies in Eq. 11 (Fig. 3). This indicates that the BDE and (IP + PDE) are perfectly correlated. The total energy requirement for the SPLET mechanism (Eq. 12, Fig. 3) is the sum of the PA (Eq. 8) and ETE (Eq. 10). Equation 12 (see Fig. 3) is the same as Eq. 11, i.e., the total energy requirements for the SPLET and SET-PT mechanisms are identical. Because the net result from all free radical scavenging mechanisms represents the difference in the enthalpy of the parent molecule and the corresponding phenoxyl radical (Fig. 3), the associated energy requirements are entirely correlated.

Tables 1 and 2 present the reaction enthalpies in water and the gas-phase, respectively, related to the active site of radical inactivation for 25 flavonoids. As previously noted, the same OH group of a particular flavonoid is the active site for radical inactivation by all three mechanisms because this OH group possess the minimal energy requirements, i.e., BDEmin, min(IP + PDE) and min(PA + ETE). The correlations of the BDE with the RSA are fair ($r$=−0.802 in water and $r$=−0.830 in the gas-phase) and are the same as correlations with the total minimal energy requirements for the SET-PT and SPLET mechanisms. Hence, as expected, the minimal energy requirements for HAT (BDE), SET-PT (IP + PDE) and SPLET (PA + ETE) are perfectly intercorrelated.

The perfect correlation of the homolytic BDE of a phenolic O–H bond (O–H BDE) with the total energy requirements related to the SET-PT and SPLET mechanisms account for the fact that the O–H BDE is generally usable as a significant descriptor that represents a radical scavenging potency of phenolic compounds such as flavonoids. Consequently, the O–H BDE may be related to any of the three free radical scavenging mechanisms due to their identical thermodynamics. This accounts for the prevalent correlation of the BDE with the radical scavenging ability of polyphenols in polar solvents [42], despite the fact that it is an unexpected result [43]. It should be noted that the strong correlation with the BDE does not implicate HAT as the thermodynamically preferred mechanism. To ascertain which radical scavenging pathway is favourable, besides the BDEs, the values of IP and PA (ETE) should also be considered [9, 19, 38].

Because the correlation between BDE of neutral flavonoid molecules and the observed experimental activity was not pronounced, we investigated the capacity of flavonoid phenoxide anions. In polar solvents the most acidic OH group of flavonoids (mainly 7-OH group) is deprotonated, and at a physiological pH of 7.4 flavonoid phenoxide anions react with free radicals [29]. Energetics related to free scavenging potency of flavonoid phenoxide anions, performed by COSMO calculations in water, are presented in Table 3.

In comparison with corresponding calculations for neutral molecules, the results obtained for phenoxide anions possess slightly better statistical characteristics. The correlation coefficient (and standard error of estimate) of BDE and ETE vs antiradical activity increases from $r$=−0.802 to $r$=−0.837, and from $r$=−0.648 to $r$=−0.725, respectively. The standard error of estimate decreases from $s$=23.01 to $s$=20.14, and from $s$=29.33 to $s$=25.34, for BDE and ETE, respectively (Tables 1, 3).

Anouar et al. [16] emphasized possibility of second HAT mechanism [23, 25], i.e., the ability of Fl–O• radical, formed after a first HAT pathway, to react with free radicals (e.g., DPPH). They found that the product formed after the second HAT is more stable, and that two HATs are more feasible. Recently, enthalpy changes associated with abstraction of two hydrogen atoms from the flavonoids were studied and

HAT
$$\mathbf{BDE} = \boldsymbol{H}(\mathbf{Fl–O^{\bullet}}) + H(\mathrm{H}) - \boldsymbol{H}(\mathbf{Fl–OH}) \quad (2)$$

SET-PT
$$\mathbf{IP + PDE} = H(\mathrm{Fl–OH^{\bullet+}}) + H(\mathrm{e^-}) - H(\mathrm{Fl–OH}) + H(\mathrm{Fl–O^{\bullet}}) + H(\mathrm{H^+}) - H(\mathrm{Fl–OH^{\bullet+}})$$
$$= \boldsymbol{H}(\mathbf{Fl–O^{\bullet}}) + H(\mathrm{e^-}) + H(\mathrm{H^+}) - \boldsymbol{H}(\mathbf{Fl–OH}) \quad (11)$$

SPLET
$$\mathbf{PA + ETE} = H(\mathrm{Fl–O^-}) + H(\mathrm{H^+}) - H(\mathrm{Fl–OH}) + H(\mathrm{Fl–O^{\bullet}}) + H(\mathrm{e^-}) - H(\mathrm{Fl–O^-})$$
$$= \boldsymbol{H}(\mathbf{Fl–O^{\bullet}}) + H(\mathrm{H^+}) + H(\mathrm{e^-}) - \boldsymbol{H}(\mathbf{Fl–OH}) \quad (12)$$

**Fig. 3** Total energy requirements for SET-PT and SPLET are the same, and the energy required for HAT is perfectly correlated with them

**Table 3** COSMO PM6 reaction enthalpies ($kJ\,mol^{-1}$) in water for 22 flavonoid phenoxide anions

| | Flavonoid | Anion site | BDE | IP | PDE | IP + PDE | PA | ETE | PA + ETE | RSA |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Morin | 3-$O^-$/2′-$O^-$ | 306.02 | 304.33 | 1.03 | 305.36 | 51.22 | 254.14 | 305.36 | 96.5 |
| 2 | Taxifolin | 7-$O^-$ | 307.80 | 369.96 | −62.82 | 307.14 | 44.86 | 262.28 | 307.14 | 94.8 |
| 3 | Kaempferol | 7-$O^-$ | 303.89 | 369.10 | −65.86 | 303.24 | 55.47 | 247.77 | 303.24 | 93.5 |
| 4 | Fustin | 7-$O^-$ | 305.86 | 343.27 | −38.06 | 305.21 | 45.08 | 260.12 | 305.20 | 91.9 |
| 5 | Galangin | 7-$O^-$ | 303.19 | 369.72 | −67.18 | 302.54 | 50.35 | 252.18 | 302.53 | 91.8 |
| 6 | Rutin | 7-$O^-$ | 301.48 | 372.21 | −71.38 | 300.83 | 37.69 | 263.14 | 300.83 | 90.9 |
| 7 | Quercetin | 7-$O^-$ | 296.55 | 369.50 | −73.60 | 295.90 | 35.35 | 260.55 | 295.90 | 89.8 |
| 8 | Luteolin 7-glucoside | 4′-$O^-$ | 303.92 | 289.31 | 13.95 | 303.26 | 88.62 | 214.64 | 303.26 | 87.6 |
| 9 | Quercetin 3-glucoside-7-rhamnoside | 4′-$O^-$ | 300.87 | 286.05 | 14.15 | 300.20 | 93.11 | 207.10 | 300.21 | 86.8 |
| 10 | Laricytrin | 7-$O^-$ | 287.68 | 373.67 | −86.64 | 287.03 | 47.21 | 239.81 | 287.02 | 84.6 |
| 11 | Laricytrin 3′-glucoside | 7-$O^-$ | 295.68 | 371.83 | −76.80 | 295.03 | 37.36 | 257.67 | 295.03 | 83.8 |
| 12 | Robinetin | 7-$O^-$ | 289.76 | 346.34 | −57.24 | 289.10 | 41.83 | 247.27 | 289.10 | 82.3 |
| 13 | Fisetin | 7-$O^-$ | 296.72 | 346.80 | −50.74 | 296.06 | 37.79 | 258.27 | 296.06 | 79.0 |
| 14 | Myricetin | 7-$O^-$ | 289.47 | 369.42 | −80.60 | 288.82 | 40.10 | 248.72 | 288.82 | 72.8 |
| 15 | Kaempferol 3,7-dirhamnoside | 4′-$O^-$ | 319.36 | 296.64 | 22.06 | 318.70 | 34.47 | 284.23 | 318.70 | 70.6 |
| 16 | Apigenin 7-glucoside | 4′-$O^-$ | 380.62 | 311.85 | 68.11 | 379.96 | 38.88 | 341.08 | 379.96 | 34.8 |
| 17 | Hesperetin | 7-$O^-$ | 307.01 | 360.66 | −54.30 | 306.36 | 57.56 | 248.79 | 306.35 | 30.0 |
| 18 | Vitexin | 7-$O^-$ | 367.80 | 360.91 | 6.24 | 367.15 | 70.39 | 296.76 | 367.15 | 21.0 |
| 19 | Naringenin | 7-$O^-$ | 355.09 | 360.34 | −5.90 | 354.44 | 66.68 | 287.76 | 354.44 | 6.3 |
| 20 | Naringin | 4′-$O^-$ | 372.69 | 282.43 | 89.60 | 372.03 | 39.16 | 332.88 | 372.04 | 4.7 |
| 21 | Chrysin | 7-$O^-$ | 361.43 | 365.59 | −4.81 | 360.78 | 63.48 | 297.29 | 360.77 | 1.1 |
| 22 | Apigenin | 7-$O^-$ | 340.40 | 364.86 | −25.11 | 339.75 | 36.78 | 302.97 | 339.75 | 0.7 |
| | | | $r$=−0.837<br>$s$=20.14 | $r$=0.024<br>$s$=36.77 | $r$=−0.524<br>$s$=31.33 | $r$=−0.837<br>$s$=20.14 | $r$=−0.068<br>$s$=36.69 | $r$=−0.725<br>$s$=25.34 | $r$=−0.837<br>$s$=20.14 | |

**Table 4** COSMO PM6 BDE values (kJmol$^{-1}$) calculated in water for the first (BDE) and second ($BDE_D$) HAT mechanism for 18 phenoxide anions of flavonoids. $BDE_{av}$ denotes average value for double HAT mechanism

| | Flavonoid | Anion site | BDE | $BDE_D$ | $BDE_{av}$ | RSA |
|---|---|---|---|---|---|---|
| 1 | Morin | 3-O$^-$/2′-O$^-$ | 306.02 | 313.59 | 309.81 | 96.5 |
| 2 | Taxifolin | 7-O$^-$ | 307.80 | 287.07 | 297.44 | 94.8 |
| 3 | Kaempferol | 7-O$^-$ | 303.89 | 308.13 | 306.01 | 93.5 |
| 4 | Fustin | 7-O$^-$ | 305.86 | 288.61 | 297.24 | 91.9 |
| 5 | Galangin | 7-O$^-$ | 303.19 | 395.22 | 349.21 | 91.8 |
| 6 | Rutin | 7-O$^-$ | 301.48 | 295.55 | 298.52 | 90.9 |
| 7 | Quercetin | 7-O$^-$ | 296.55 | 303.13 | 299.84 | 89.8 |
| 8 | Luteolin 7-glucoside | 4′-O$^-$ | 303.92 | 308.41 | 306.17 | 87.6 |
| 9 | Quercetin 3-glucoside-7-rhamnoside | 4′-O$^-$ | 300.87 | 302.34 | 301.61 | 86.8 |
| 10 | Laricytrin | 7-O$^-$ | 287.68 | 302.58 | 295.13 | 84.6 |
| 11 | Laricytrin 3′-glucoside | 7-O$^-$ | 295.68 | 315.73 | 305.71 | 83.8 |
| 12 | Robinetin | 7-O$^-$ | 289.76 | 305.23 | 297.50 | 82.3 |
| 13 | Fisetin | 7-O$^-$ | 296.72 | 301.85 | 299.29 | 79.0 |
| 14 | Myricetin | 7-O$^-$ | 289.47 | 311.60 | 300.54 | 72.8 |
| 15 | Hesperetin | 7-O$^-$ | 307.01 | 427.18 | 367.10 | 30.0 |
| 16 | Vitexin | 7-O$^-$ | 367.80 | 403.10 | 385.45 | 21.0 |
| 17 | Naringenin | 7-O$^-$ | 355.09 | 355.82 | 355.46 | 6.3 |
| 18 | Apigenin | 7-O$^-$ | 340.40 | 499.60 | 420.00 | 0.7 |
| | | | $r$=−0.804<br>$s$=19.79 | $r$=−0.803<br>$s$=19.84 | $r$=−0.875<br>$s$=16.12 | |

related to the experimental activities of the flavonoids [44]. Our results of COSMO calculations in water for the first and second HAT (BDE and $BDE_D$, respectively) for flavonoid phenoxide anions are presented in Table 4. Considered flavonoids possess at least three phenolic OH groups. Average BDE value of two HATs is denoted by $BDE_{av}$ [16]. By using $BDE_{av}$ as a descriptor we obtained $r$= −0.875 and $s$=16.12. Excluding two outliers (galangin and naringenin) from the data set, the statistical analysis results in a one-descriptor model of good quality (Eq. 13):

$$\begin{aligned} &\mathrm{RSA} = 313(\pm 17.501) - 0.752(\pm 0.055)\mathrm{BDE_{av}} \\ &N = 16 \quad r = --0.965 \quad s = 7.98 \quad F = 189.29 \end{aligned} \tag{13}$$

In Eq. 13, $N$ represents the number of compounds, $r$ is the correlation coefficient, $s$ the standard error of estimate, and $F$ is Fisher $F$-value. Figure 4 plots experimental RSA values versus $BDE_{av}$ for 16 flavonoids listed in [15]. Linear regression model presented by Eq. 13 outperforms our two-descriptor models developed on the same data set [45, 46]. This indicates high potential of $BDE_{av}$ as a descriptor for modeling flavonoids RSA.

As can be seen from Table 4, morin and hesperetin possess nearly identical BDE values (306.02 vs 307.01 kJ mol$^{-1}$) and very different RSA (96.5 % vs 30 %). Corresponding $BDE_{av}$ values of 309.81 vs 367.10 kJmol$^{-1}$ for morin and hesperetin, respectively, indicating that a second HAT mechanism for hesperetin is much less probable than for morin. Considering the chemical structure of hesperetin, this result is expected due to the lack of structural requirements for effective radical scavenging, i.e., vicinal OH groups in B ring, C-3 phenolic OH group and C2–C3 double bond in C ring [47]. The lower activity of hesperetin vs morin is related to the second HAT mechanism ($BDE_D$), and not to the first HAT mechanism (BDE). This fact approves usefulness of $BDE_D$ and $BDE_{aw}$ as

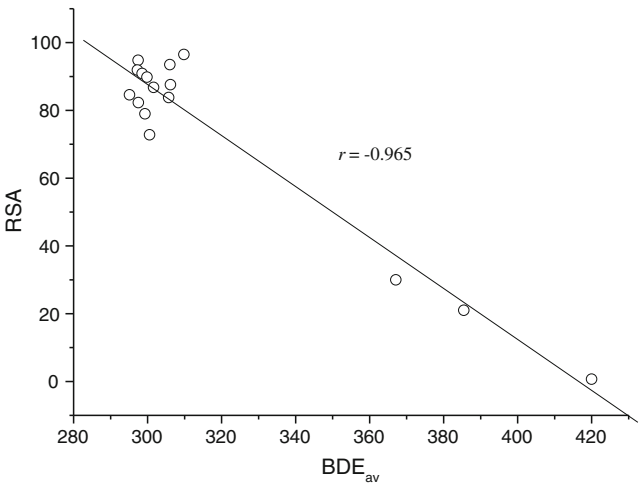



**Fig. 4** Scatter plot of experimental RSA values obtained by the DPPH radical versus calculated $BDE_{av}$ for data set of 16 flavonoids from [15]

descriptors related to free scavenging activity of phenolic compounds.

## Conclusions

In this report, we showed that the BDE, which is by definition related to the HAT mechanism, can also be associated with the SET-PT and SPLET mechanisms. This is so because the total energy requirements related to the SET-PT (sum IP and PDE) and SPLET (sum PA and ETE) mechanisms are equivalent to those of the HAT mechanism and are correlated perfectly with the BDE values. The HAT mechanism leads to the formation of the same products as those obtained by SET-PT and SPLET mechanisms and all three mechanisms are thermochemically identical. This could be a major reason why the published data on polyphenolic antioxidant activities measured by various assays correlate better with the BDE than with other reaction enthalpies involved in radical scavenging mechanisms, i.e., IP, PDE, PA and ETE. The BDE is able to fairly rank flavonoids as antioxidants in any medium, but to determine which radical scavenging mechanism represents the most probable reaction pathway from the thermodynamic point of view, the IP and PA (ETE) should also be considered. Consideration of the second HAT mechanism and the use of $BDE_{av}$ as a descriptor for modeling RSA of flavonoids is confirmed.

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