Perturbations of an Acetone-Based *Briggs–Rauscher* System by α-Tocopherol (Vitamin E)

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The malonic acid (MA)-based oscillating *Briggs–Rauscher* reaction (*BR*) in batch mode has been shown to be sensitive to various hydrophilic polyphenol antioxidants. Several of these have been shown to cause cessation of oscillations for a period of time before a restart occurs. The length of time before oscillations restart is related to the type of antioxidant and its concentration. Procedures have been devised to use this method as a tool for measuring antioxidant activity from pure compounds and from extracts of natural sources. The antioxidant activity has been related to the reaction of the antioxidants with HOO• radicals present in the oscillating system. Vitamin E (α-tocopherol), a typical highly lipophilic antioxidant containing an phenolic OH group, is soluble in acetone that also is a suitable substrate for the *BR* reaction. Perturbations of a highly concentrated acetone-based *BR* oscillator by acetonic solutions of vitamin E were studied. The inhibitory effects were found similar to those provoked by hydrophilic polyphenols in the MA-based oscillator, but to obtain reasonable inhibition times, the concentration of vitamin E must be at the mM level instead μM. However, there is a region of concentrations where there is a nearly linear relation between concentration and inhibition time. A comparison with a hydrophilic diphenol (2,6-dihydroxybenzoic acid) in the acetone-based oscillator showed that the inhibitory reaction is much slower in this system than in the MA one. We were able to model the perturbations by vitamin E assuming its reaction with HOO• radicals by using the *FCA* mechanism previously reported with some little modifications.

1. Introduction. – Inhibitory effects by polyphenolic antioxidants on the oscillations of a malonic acid-based *Briggs–Rauscher* (*BR*) system [1] have been extensively investigated in acidic aqueous and mixed EtOH/H_2O media [2–5]. These inhibitory effects consist of an immediate quenching of oscillations, an inhibition time that linearly depends on the activity and concentration of the antioxidant added in a wide range of concentration, and subsequent regeneration of oscillations [2–4]. These effects were ascribed to the removal of hydroperoxy radical, HOO• (an important intermediate of the *BR* system) by the phenolic OH group(s) of the antioxidant. Mechanistic calculations supported this interpretation [3][5]. All this enabled us to implement a test to measure the relative antioxidant activity of polyphenols [3]. This test, called the *BR* method, was found effective in assessing the antioxidant power of natural polyphenolic compounds [6][1]) and plant extracts [7]. The *BR* method was

[1]) Also mentioned in the February 17, 2003 issue of *Gastroenterology Week*.

successfully used in independent laboratories [8][9] and was included in a review on antioxidant methods [10][2]). However, the malonic acid-based *BR* method is effective only on hydrophilic or EtOH/H_2O (20% (*v*/*v*)) mixed soluble antioxidants.

Acetone is an elective solvent for strongly lipophilic antioxidant free-radical scavengers as vitamin E (α-tocopherol), and it also is a good organic substrate for the *BR* reaction instead of malonic acid and its C(2) substituted derivatives. The acetone-based *BR* system was first investigated by *Cooke* in the [acetone] range 0.20–6.0M [12][13], and then revised by *Furrow* in the [acetone] range 0.27–2.76M [14]. Some attempts were made by these authors in qualitatively interpreting some behaviour differences between the two substrates, but apart the reaction between acetone and H_2O_2 to give adducts as 2-hydroxy-2-hydroperoxypropane (HP1) and 2,2-bis(hydroperoxy)propane (HP2), *Furrow* claimed that the initial behaviour of the *BR* oscillator in the batch mode is rather similar for malonic acid and acetone [14]. On these basis, we decided to investigate the inhibitory effects by α-tocopherol (vitamin E) on the oscillations of the H_2O_2/acidic iodate/manganese(II)/acetone oscillating system. The possibility of implementing a *BR* method to assess the relative antioxidant properties, taking α-tocopherol as a standard, was also outlined.

2. Experimental. – *Materials and Methods.* Malonic acid (*Merck*; reagent grade, >99%), $MnSO_4 \cdot H_2O$ (*Merck*; reagent grade, >99%), $NaIO_3$ (*Merck*; reagent grade, >99.5%), acetone (*Fluka*; reagent grade >99.5%), and α-tocopherol (vitamin E, *Aldrich*; 96%) were used without further purification. $HClO_4$ (*Merck*; 70–72%) and H_2O_2 (*Merck*; 35–36.5%) were of analytical grade. $HClO_4$ was analyzed by titration *vs.* a standard 0.1M NaOH soln. (from *Merck*). H_2O_2 was standardized daily by manganometric analysis. Iodic acid (HIO_3) 99.5% (*Alfa Aesar*) was used as received. Solid I_2 (*Merck*; reagent grade 99.8%) was stirred in doubly distilled H_2O for several h. The soln. was filtered through *Pyrex* wool and stored in a glass-stoppered bottle protected from light. Antioxidants used: 2,6-DHBA (=2,6-dihydroxybenzoic acid, *Aldrich*; reagent grade, 98%), resorcinol (=benzene-1,3-diol, *Fluka*; reagent grade ≥98%).

Oscillations in the *BR* mixtures were followed potentiometrically either by recording the potential of the soln. with a bright-Pt electrode (*Hamilton*, model *P/N 238945*) – reference electrode (double-junction Ag/AgCl electrode, *Ingold*, model *373-90-WTE-ISE-S7*), or the potential of an iodide-ion-selective electrode (*Orion*, model *9453*) – reference electrode couple. In Germany, potentiometric measurements were made with a combined redox electrode (*Mettler*, *Toledo InLab 501*). Electrodes were connected to a pH-multimeter (*WTW*, model pH *540 GLP*) controlled by an *IBM*-compatible PC. The accuracy of the multimeter was ±1 mV. The data-acquisition program Multi Achat II (*WTW*) was used. The multimeter was equipped with a temp. sensor with an accuracy of ±0.1°.

The kinetics of the subsystems (vitamin E + IO_3^- + H^+ in acetonic medium) and (vitamin E + H_2O_2 in acetonic medium) were followed spectrophotometrically by using a *Shimadzu* model *UV-1601PC* spectrophotometer equipped with thermostated cell compartments and magnetic microstirrer. The program, UVPC by *Shimadzu*, was used. The temp. was maintained constant at 25.0±0.1°. Mixtures were prepared directly in a 1.000 cm optical path quartz cell by mixing the appropriate amounts of stock solns. of reagents using precision micropipettes. Recordings started immediately after the addition of the last reagent.

A possible reaction between iodoacetone and antioxidants in aqueous acidic medium was investigated by ^{1}H- and ^{13}C-NMR on a 300 MHz *Bruker Avance Microbay H-X ATM* broadband probe and a deuterium-lock channel at r.t.

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[2]) Perturbations of the oscillatory regime of the *BR* reaction have also been observed by the addition of ascorbic acid (vitamin C), but the mechanism of its action is primarily due to interaction with iodine intermediates in the V, III, I, and 0 oxidation states to give iodide ions [11].

Preparation of Stock Solns. for the BR *Experiments. 1*) Aq. acetone 13.5M: to prepare 100 ml of this soln., H_2O was added to 99.3 ml acetone >99.5% to 100 ml final volume; *2*) H_2O_2 3.6M in acetone: to prepare 100 ml of this soln., 69 ml acetone >99.5% were gently added to 31 ml of H_2O_2 (35% (*v*/*v*) exactly titred). ***Caution!*** Acetone and H_2O_2 form a potential explosive mixture. It is prudent to operate in an ice bath using protective glasses and gloves; *3*) aq. iodate and perchloric acid soln.: 0.06M $NaIO_3$, 0.30M $HClO_4$; *4*) Aq. soln. of manganese(II) sulfate: 0.030M with reference to $MnSO_4 \cdot H_2O$; all the aq. solns. were prepared from doubly distilled, deionized H_2O; *5*) Acetone soln. of α-tocopherol: 1.0 g of vitamin E was dissolved in acetone in a 10.0 ml volumetric flask, and then, acetone was added to volume. This soln. is 0.232M regarding α-tocopherol[3]). Since, as stated above, acetone and H_2O_2 react to give the adducts HP1 and HP2, it is convenient to prepare the suitable amount of soln. *2* immediately before each experiment.

Preparation of the Blank Mixture (31 ml). Using micropipets or pipets, in the following order: 8.0 ml of soln. *1*, 10.0 ml of soln. *2*, 10.0 ml of soln. *3*, 1.00 ml of acetone, and 2.0 ml of soln. *4* were mixed. The nominal initial concentrations of this mixture were: $[Ac]_{tot} = 7.0$M; $[H_2O_2] = 1.16$M; $[IO_3^-] = 0.0194$M; $[HClO_4] = 0.097$M; $[Mn^{II}] = 0.00194$M. Oscillations in this mixture started immediately after the addition of soln. *4*.

Preparation of a Perturbed BR *Mixture.* Instead of 1.0 ml of acetone, 1.0 ml of soln. *5* was added. Oscillations started only after some time, determined by the inhibition due to vitamin E. The nominal initial concentrations of this mixture was as above plus $[\text{vit. E}] = 7.5 \times 10^{-3}$ M.

In order to limit the loss of acetone during the recording, the electrodes were inserted in suitable holes made in an aluminium foil fixed to the top of the reaction vessel (a 100 ml beaker).

A problem arose with thermostatation because after the addition of acidic iodate to the reactive mixture the temp. rised a few degrees in spite of the thermostat, initially 25.0°. To maintain the temp. constant, a delicate interplay between the thermostat and the stirrer-heater was necessary. We often operated at 26.0 ± 0.5°.

3. Results. – *Behavior of the Completely Unperturbed and Perturbed Oscillator.* Recordings of V(Pt) *vs.* time for a blank and a typical mixture perturbed by α-tocopherol (6.74 mM) are reported in *Fig. 1*.

In *Fig. 2*, the fitted straight line t_{inhib} *vs.* concentration of α-tocopherol is shown.

As can be seen from *Figs. 1* and *2*, the concentration of vitamin E in the mixture has to be at mM level in order to obtain reasonable inhibition times, while for hydrophilic antioxidants in the malonic acid-based *BR* oscillator, very high inhibition times were obtained at μM level [2–5][4]).

The first part of a graph of $\log[I^-]$ *vs.* time for an acetone-based *BR* reaction is reported in *Fig. 3*. This was obtained by using the couple iodide-selective electrode/reference electrode (see *Experimental*), suitably transforming $V(I^-)$ into $\log[I^-]$ by means of a calibration curve $V(I^-)$ *vs.* $\log[I^-]$.

Although there are slight differences in the oscillation period and in the induction time, the shape of $\log[I^-]$ *vs.* time is similar to that in the malonic acid-based aqueous [2] and mixed ($EtOH/H_2O$) [3] medium. This strongly suggests that the main features of the reaction mechanism in the acetone-based *BR* reaction under batch conditions are the same as those for the malonic acid-based oscillator.

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[3]) α-Tocopherol at concentrations that give inhibitory effects is soluble only at high acetone concentration.

[4]) Similar results were obtained in Germany using the combined redox electrode: couples [vit. E] [mM], t_{inhib} [s]: 6.74, 154; 5.99, 133; 5.24, 107; 4.49, 81; 3.75, 62. This confirms the reproducibility of the experiment.

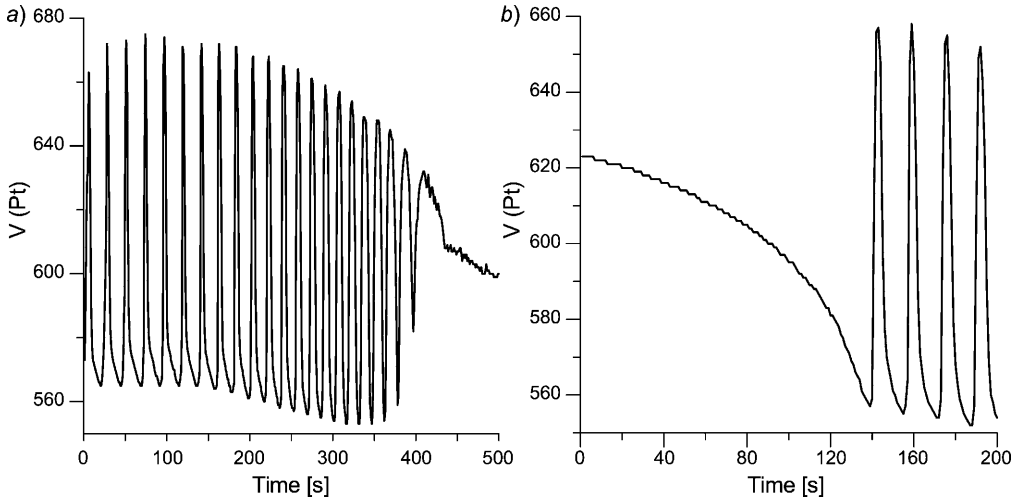

Fig. 1. *a*) *Recording of V(Pt)* vs. *time for the acetone-based BR oscillator.* Nominal initial concentrations in mixture: $[Ac]_{tot} = 7.0$M; $[H_2O_2] = 1.16$M; $[IO_3^-] = 0.0194$M; $[HClO_4] = 0.097$M; $[Mn^{II}] = 0.00194$M; *b*) *Recording of V(Pt)* vs. *time for the oscillator perturbed by vitamin E* (6.74 mM).

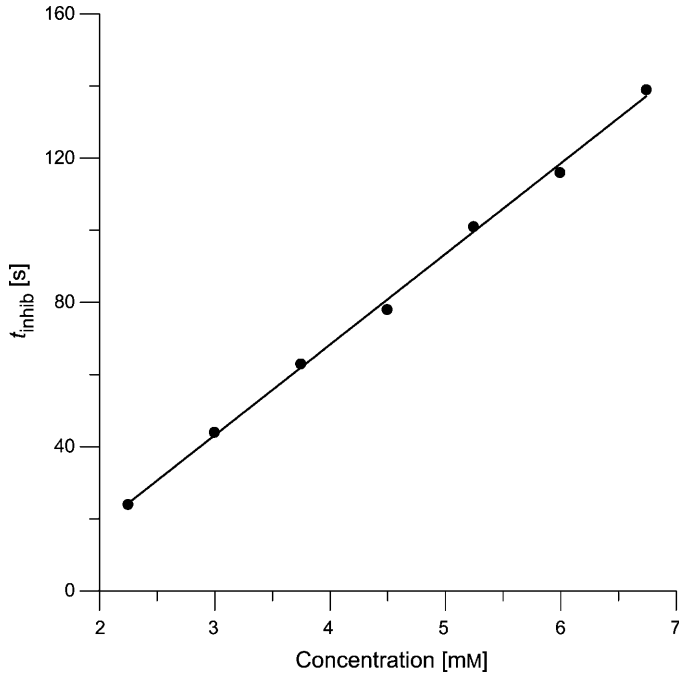

Fig. 2. *Graph of the inhibition time* vs. *[vitamin E] in the acetone-based* BR *mixture*

Comparison Between the Effects of a Hydrophilic Antioxidant in the Acetone- and Malonic Acid-Based Systems. For this comparison, we chose 2,6-DHBA as antioxidant,

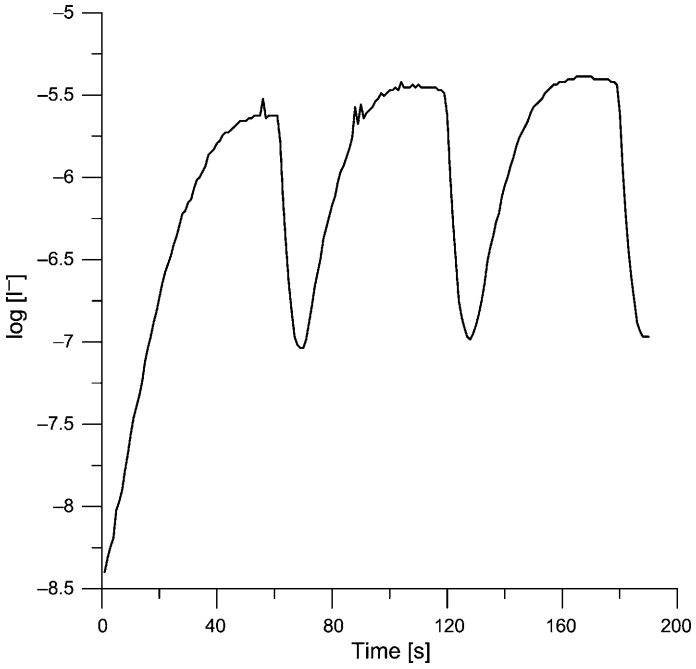

Fig. 3. *Graph of log[I⁻]* vs. *time for an acetone-based* BR *reaction*. Nominal initial concentration in mixture: $[Ac]_{tot} = 3.2$M; $[H_2O_2] = 1.5$M; $[IO_3^-] = 0.02$M; $[HClO_4] = 0.10$M; $[Mn^{II}] = 0.002$M.

since this compound has been found the most suitable standard for polyphenolics in matrices [15].

In *Fig. 4*, the straight lines t_{inhib} *vs.* [2,6-DHBA] in the two systems are reported.

As can be seen, the inhibition times in the acetone-based system are considerably shorter than those in the aqueous malonic acid system. However, the concentration of 2,6-DHBA that gives 163 s inhibition time in the acetone system is about two orders of magnitude higher than that giving 394 s in the malonic acid system. This is an indication that the step IN [5]:

$$ArOH + HOO^{\bullet} \rightarrow ArO^{\bullet} + H_2O_2$$

(where ArOH indicates a generic phenolic antioxidant) is considerably slower in the acetone-based *BR* oscillator. Similar results were found for resorcinol, a standard used for *BR* antioxidant activity measurements on pure compounds [3][6].

Kinetics of the Subsystem Vitamin E + Acidic Iodate in Acetone/H_2O. We prepared a solution of 15 mg of vitamin E in 15 ml of acetone and a solution of 2 mg of $NaIO_3$ plus 0.5 ml of 72% $HClO_4$ to a volume of 10 ml with distilled H_2O. The two solutions were rapidly mixed, and an aliquot was put into a cuvette. Spectra from 550 nm to 300 nm were recorded at different times (10, 15, 20, up to 55 min) *vs.* a vitamin E/acetone/H_2O blank. In the spectra with iodate, in the region around 320 nm, a decline of absorption was observed. If neither iodate nor vitamin E are responsible for this effect, it should be

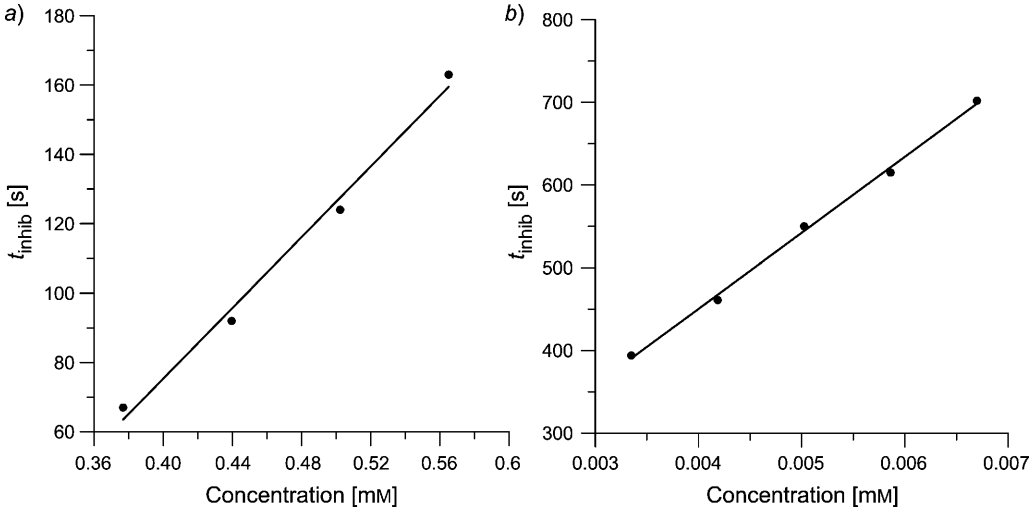

Fig. 4. *a*) *Straight line* t_{inhib} vs. *[2,6-DHBA] mM in the acetone-based system*; *b*) *straight line* t_{inhib} vs. *[2,6-DHBA] mM in the malonic acid-based system*

an intermediate. The quinone has a major absorbtion band at 265 nm and a small shoulder at *ca.* 300 nm, and a very minor band around 350. The first two bands are completely hidden by the strong absorbance of acetone. Oxidized vitamin E has another broad absorbance near 460 nm. After 55 min, a very weak absorbance appeared around 450 nm. These data are so far consistent with oxidation of vitamin E to an unknown intermediate, but are not a substantial proof.

Subsystem Acetone/H_2O_2/H_2O. Spectra of an acetone/H_2O reference blank and an acetone/H_2O_2/H_2O mixture prepared as reported above showed an absorbtion band around 320 nm. There is a steady decrease of absorbtion intensity with time in this wavelength region. Below *ca.* 316 nm, an almost total absorbance is observed in the blank sample which causes the band. We believe the drop of absorbtion intensity is related to the formation of the acetone-peroxide adduct.

Investigation of the Subsystem Iodoacetone + Antioxidants in Acetone/H_2O. In order to explore the possibility that iodoacetone reacts with antioxidants, iodoacetone was prepared *in situ* mixing 3.0 ml of acetone, 2.0 ml of H_2O, 0.512 g of ground I_2, and 0.183 g of HIO_3. This mixture was allowed to stir overnight until it was clear. In both ^{1}H- and ^{13}C-NMR, signals for acetone and iodoacetone were detected as expected. No multi-iodoacetone peaks were observed. A mixture was then prepared according to the following: 0.099 g of resorcinol, 0.40 ml of H_2O, 0.05 ml of $HClO_4$ (2.0M), and 0.20 g of iodoacetone/acetone mixture from above. NMR Spectra were taken within about 10 to 25 min after mixing. Both ^{1}H- and ^{13}C-NMR showed acetone, iodoacetone, and resorcinol. No sign of any iodoresorcinol or any other new product was observed. These results are evidence that iodoacetone is an end product and not an intermediate in the acetone-based *BR* oscillator.

4. Theory. – In order to simulate the experimental behaviour, the model reported in *Table 1* was used.

Table 1. *The Model Used in the Simulations* [5][16]

Step	Equation	Rate constant	Ref.
D1	$HOI + H_2O_2 \rightarrow H^+ + I^- + O_2$	20 $M^{-1}\,s^{-1}$	this work
M	$HOIO + H_2O_2 + Mn^{2+} + H^+ \rightarrow HOI + Mn^{3+} + HOO^\bullet + H_2O$	6.0×10^5 $M^{-2}\,s^{-1}$	this work
Mb	$H_2O_2 + Mn^{3+} \rightarrow Mn^{2+} + HOO^\bullet + H^+$	1.0×10^4 $M^{-1}\,s^{-1}$	this work
D2	$H^+ + IO_3^- + HOO^\bullet \rightarrow IO_2^\bullet + O_2 + H_2O$	1.0×10^4 $M^{-2}\,s^{-1}$	this work
D3	$IO_2^\bullet + H_2O_2 \rightarrow HOIO + HOO^\bullet$	35 $M^{-1}\,s^{-1}$	this work
I5R	$2\,IO_2^\bullet + H_2O \rightarrow H^+ + IO_3^- + HOIO$	6.0×10^8 $M^{-1}\,s^{-1}$	this work
O2	$2\,HOO^\bullet \rightarrow H_2O_2 + O_2$	7.5×10^5 $M^{-1}\,s^{-1}$	[5]
W1	$H^+ + OH^- \rightarrow H_2O$	2×10^{10} $M^{-1}\,s^{-1}$	Diffusion controlled
C3	$Ac_{keto} + H^+ \rightleftharpoons Ac_{enol} + H^+$	$k_f = 3 \times 10^{-5}$, $k_r = 180$ $M^{-1}\,s^{-1}$	[14]
C4	$I_2 + Ac_{enol} \rightarrow H^+ + I^- + IAc$	1.0×10^7 $M^{-1}\,s^{-1}$	[14]
I2A	$I^- + HOIO + H_2O \rightarrow 2\,HOI + OH^-$	3.0×10^9 $M^{-1}\,s^{-1}$	this work
I3	$2\,H^+ + I^- + IO_3^- \rightarrow HOI + HOIO$	1.43×10^3 $M^{-3}\,s^{-1}$	[5]
I1	$HOI + I^- \rightarrow I_2 + OH^-$	3.67×10^9 $M^{-1}\,s^{-1}$	[5]
IN	$HOO^\bullet + \text{vitamin E} \rightarrow H_2O_2 + \text{vitamin E}^\bullet$	allowed to vary	this work
DEG	vitamin E $\rightarrow$ prod.	allowed to vary	this work
I1R	$I_2 + H_2O \rightarrow HOI + H^+ + I^-$	1.98×10^3 s^{-1}	[5]
A1	$H^+ + H_2O_2 + Ac \rightleftharpoons HP1 + H^+$	$k_f = 2.9$ $M^{-2}\,s^{-1}$, $k_r = 33.8$ $M^{-1}\,s^{-1}$	[14]
A2	$H^+ + HP1 \rightleftharpoons P1^+$	$k_f = 3.4$ $M^{-1}\,s^{-1}$, $k_r = 5.1 \times 10^3$ s^{-1}	[14]
A3	$H_2O_2 + P1^+ \rightleftharpoons HP2 + H^+$	$k_f = 29$ $M^{-1}\,s^{-1}$, $k_r = 5.5 \times 10^{-3}$ $M^{-1}\,s^{-1}$	[14]

Ac_{keto} = acetone in the keto form; Ac_{enol} = acetone in the enolic form; vitamin E = α-tocopherol; vitamin $E^\bullet$ = radical at the O-atom of vitamin E; HP1 = 2-hydroxy-2-hydroperoxypropane; $P1^+$ = protonated HP1; HP2 = 2,2-bis(hydroperoxy)propane.

Steps D1, M, D2, D3, I5R, O2, and I3 were taken from the *FCA* (*Furrow*, *Cervellati*, *Amadori*) mechanism that was found effective in simulating the *BR* oscillator with malonic acid and its C(2) substituted derivatives, as well as with other substrates that iodinate in a different way [16]. These steps represent the oxyiodine intermediate reactions and the formation and consumption of radicals. To this group of reaction steps, I1, I1R, and I2 were modified, and W1 was added to change the way $[H^+]$ was handled; M was split into M and Mb. Some of the values of rate constants were adjusted so that the length of oscillations in the simulated unperturbed oscillator was close to the experimental value. The adjustment reflects the fact that rate constants in the acetone/H_2O mixture are undoubtedly different from the values in H_2O as the solvent. Steps C3 and C4 represent the iodination of acetone. Steps A1 – A3 represent the reactions between acetone and H_2O_2 in acidic medium. Steps IN and DEG represent the reaction of subtraction of $HOO^\bullet$ radicals by vitamin E and the possible parallel reaction of vitamin E with acidic iodate or H_2O_2. Simulations were performed with the numerical integrator program COPASI [17]. For the perturbed oscillator, the rate constants were kept fixed to those reported in *Table 1* while the k_{IN} and k_{DEG} constants were varied for the best fit to the experimental behaviours.

In *Fig. 5*, the simulated behaviour of an acetone based *BR* oscillator with initial nominal reagent concentrations as in *Fig. 1*, *a*, is shown.

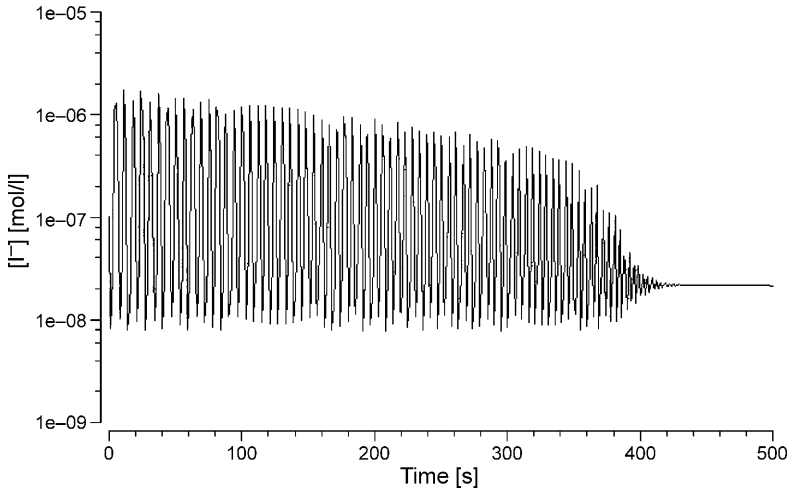

Fig. 5. *Simulated behaviour of [I^-]* vs. *time of the acetone-based* BR *oscillator* (initial nominal concentrations as in *Fig. 1*,*a*).

As can be seen, the duration of the oscillatory regime is similar to the experimental one even though the frequency of oscillations is rather different.

In *Fig. 6*, the behaviour of an acetone-based *BR* oscillator perturbed by vitamin E at the same nominal initial concentrations of *Fig. 1*,*b*, is reported.

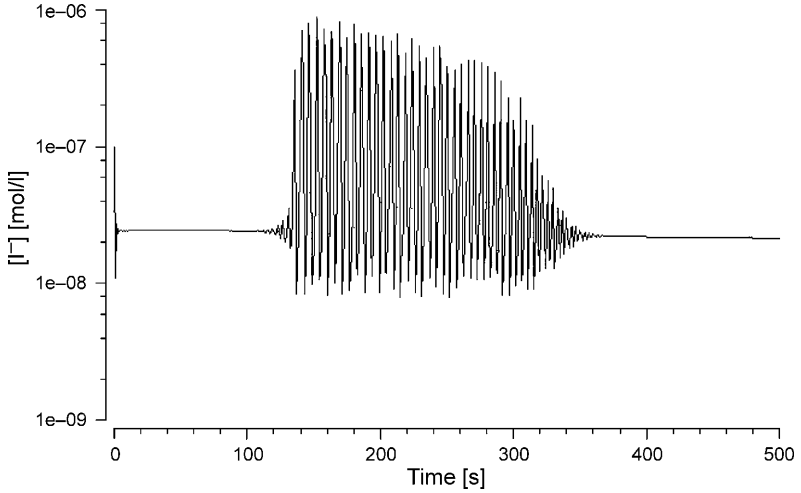

Fig. 6. *Simulated behaviour of [I^-]* vs. *time of the perturbed acetone-based* BR *oscillator by vitamin E* (initial nominal concentrations as in *Fig. 1*,*b*).

The very good agreement between the experimental (139 s) and the simulated (134 s) inhibition time can be seen. Similar agreement was found for all the

experiments (see *Table 2*)[5]), obtaining the following unique values: $k_{IN} = 750\ M^{-1}\ s^{-1}$, $k_{DEG} = 4.2 \times 10^{-2}\ s^{-1}$.

Table 2. *Experimental and Calculated Inhibition Times for an Acetone-based* BR *System Perturbed by Vitamin E*

Concentration of vitamin E [mM]	t_{inhib} exp. [s]	t_{inhib} calc. [s]
6.74	139	134
5.99	116	121
5.24	101	102
4.49	78	95
3.75	63	60
3.00	44	41
2.25	24	16

Calculations were also made on 2,6-DHBA perturbed acetone- and malonic acid-systems, adopting for the acetone-system the model reported here and for the malonic acid-system the *FCA* model described in [5][15]. The obtained results are reported in *Table 3*.

Table 3. *Experimental and Calculated Inhibition Times for* BR *Systems Perturbed by 2,6-DHBA*

2,6-DHBA in the acetone-based system			2,6-DHBA in the malonic acid-based system		
conc. [mM]	t_{inhib} exp. [s]	t_{inhib} calc. [s]	conc. [mM]	t_{inhib} exp. [s]	t_{inhib} calc. [s]
0.565	163	165	0.00670	702	690
0.502	124	126	0.00586	615	633
0.440	92	92	0.00502	550	563
0.377	67	63	0.00419	461	480
			0.00335	394	383

The good agreement between experimental and calculated inhibition times can be noted. The calculated unique values of k_{IN} and k_{DEG} are:

2,6-DHBA in the acetone-system: $k_{IN} = 5.4 \times 10^3\ M^{-1}\ s^{-1}$; $k_{DEG} = 1.9 \times 10^{-2}\ s^{-1}$

2,6-DHBA in the malonic acid-system: $k_{IN} = 3.80 \times 10^7\ M^{-1}\ s^{-1}$; $k_{DEG} = 2.2 \times 10^{-3}\ s^{-1}$

As can be seen, the value of k_{IN} for the perturbed acetone-based *BR* system is four orders of magnitude lower than that in the aqueous malonic acid-system. This is a mechanistic confirmation that the reaction of scavenging $HOO^{\bullet}$ radicals is considerably slower in the acetone-based system than in the malonic acid-based one.

5. Conclusions. – The results obtained here indicate that a very highly lipophilic phenolic substance, as for instance vitamin E, that contains a phenolic group, is able to perturb the oscillatory regime of an acetone-based *Briggs–Rauscher* oscillating system in a similar way as that found for hydrophilic antioxidants in the aqueous malonic acid-based *BR* oscillator [2–4]. Hydrophilic polyphenols, as for example 2,6-dihydroxybenzoic acid, also gave similar inhibitory effects. The inhibitory reaction, however, is

[5]) Similar agreement was obtained with the data collected in Germany; $k_{IN} = 700\ M^{-1}\ s^{-1}$, $k_{DEG} = 3.6 \times 10^{-2}\ s^{-1}$.

considerably slower in the acetone-based system: in order to obtain inhibition times similar to those observed in the aqueous malonic acid-based oscillator, the concentration of the inhibitor must be at mM instead the μM level. Experimental results were confirmed by mechanistic calculations based on the previously reported *FCA* model [5][16] with only minor modifications. In both systems, the inhibitory effects were ascribed to the scavenging action by antioxidants on the $HOO^{\cdot}$ radicals present in the *BR* systems as intermediates (see step IN in *Table 1*). However, the calculated k_{IN} value in the acetone-based oscillator is considerably smaller (order of 10^3 M^{-1} s^{-1}) than that in malonic acid-based system (order of 10^7 M^{-1} s^{-1}), in agreement with the experimental findings.

Based on the results reported here, it seems possible to implement a method to assess the relative antioxidant activity of lipophilic compounds based on an acetone-based *BR* oscillating system. This will be the object of a further work.

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