Inhibitory Effects by Ascorbic Acid on the Oscillations of the *Briggs–Rauscher* Reaction

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The *Briggs–Rauscher* oscillating reaction (in batch mode) has been shown to be sensitive to various antioxidants, some of which 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 apply this method as a tool for measuring antioxidant activities of pure compounds and of extracts of natural sources. The antioxidant activity in the *Briggs–Rauscher* system has been generally related to the reaction of an antioxidants with hydroperoxy radicals ($HOO^{\bullet}$) present in the oscillating system. Thereby, at low concentration ($< 2 \times 10^{-4}$ M), ascorbic acid is known to have a little effect on the reaction. However, there is a concentration range, where a nearly linear relation is observed between ascorbic acid concentration and inhibition time. We were able to model this type of inhibition by the reducing power of ascorbic acid without invoking a reaction with $HOO^{\bullet}$.

1. Introduction. – We have published a series of papers [1–4] concerning the inhibitory effects of antioxidants on the *Briggs–Rauscher* (*BR*) oscillating reaction [5]. The visually striking *BR* oscillating system, containing acidic iodate, hydrogen peroxide, manganous ions, and malonic acid (or another appropriate organic substrate) changes color from clear to yellow to blue (when starch is present), and then repeats this sequence several times per minute for several minutes in the batch mode. Phenolic antioxidants can cause cessation of the oscillations for a length of time proportional to the antioxidant concentration [1–4]. This method has been suggested as a test for antioxidant activity in solutions comparable to the acidity of the human stomach [2][6].

Modelling studies using the *FCA* (*Furrow–Cervellati–Amadori*) model [7] have been successful in simulating the variable inhibition periods based on reaction of the intermediate hydroperoxy radical ($HOO^{\bullet}$) with the various antioxidants. Although ascorbic acid (ASC) is no phenol, it was of interest to compare its effect with that of other antioxidants.

2. Results. – 2.1. Briggs–Rauscher *Oscillation with Ascorbic Acid*. The reference oscillating system was made from 50 mM malonic acid (MA), 6.7 mM $MnSO_4$, 26.6 mM $HClO_4$, 66.7 mM $NaIO_3$, and 1.2M H_2O_2, added in this order. After the third oscillation, ascorbic acid (ASC) solution (1 ml) was added to the oscillating solution (30 ml). At an ASC concentration of *ca.* 2×10^{-4} M in the final mixture, there was no appreciable

inhibition, and little effect on the amplitude or period, whereas other antioxidants were inhibitory already in the micromolar concentration range. Typical unperturbed runs are shown in *Fig. 1*, as determined by measuring the electromotive force with a bright platinum electrode relative to a reference electrode.

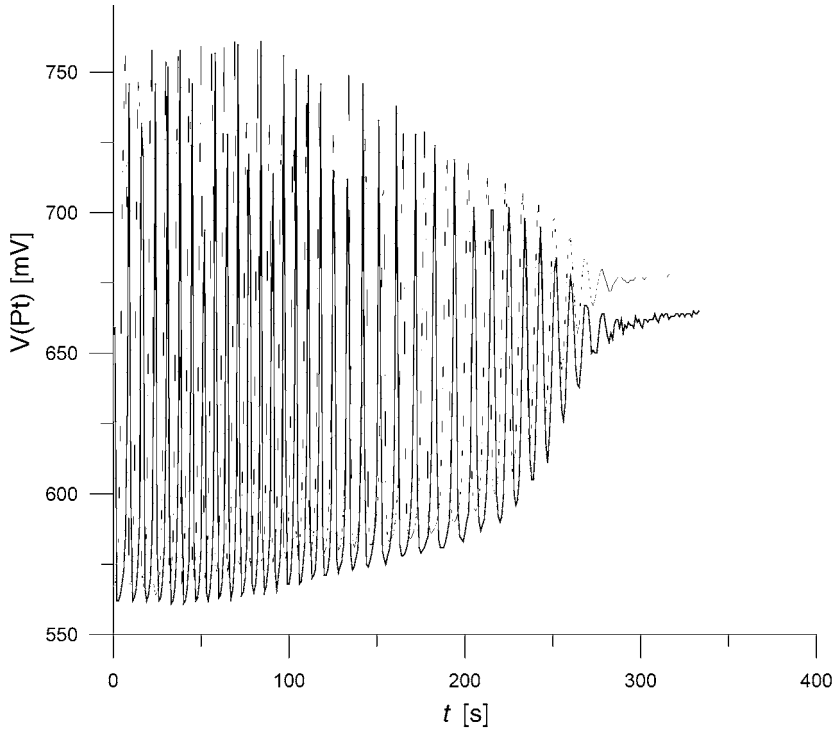

Fig. 1. *Comparison of two unperturbed runs of the* Briggs–Rauscher *oscillation in the absence and presence of ascorbic acid* (ASC). The darker line, at lower potential, represents the experiment performed without ASC, the lighter line that with ASC (1.8×10^{-4} M). ASC was added after the third oscillation. The oscillations were followed potentiometrically (see *Exper. Part*).

Above a concentration of ASC of *ca.* 2 mM in the mixture, there was an immediate cessation of oscillations upon addition of more ASC. The inhibition time is defined as the time during which the oscillation is interrupted. For example, when 1 ml of a stock solution of 50 mg/ml of ASC was added to 30 ml of the reference oscillating solution (see above), giving rise to [ASC] = 9.16 mM, then an inhibition time of 40 s was observed, as shown in *Fig. 2*.

In a concentration range of *ca.* 2–14 mM of ASC in the final mixture, the inhibition time increased linearly, as shown in *Fig. 3*. However, at 15 mM ASC concentration, there was no restart in one trial, whereas, in another, the inhibition time was 320 s.

2.2. Briggs–Rauscher *Oscillation with KI.* We have proposed that previously used antioxidants interact with the *BR* oscillator *via* reaction with HOO$^{\bullet}$ radicals [1–4]. ASC, however, is capable of reducing iodine compounds to iodide. The amounts added to achieve inhibition are large enough that significant concentations of iodide can be

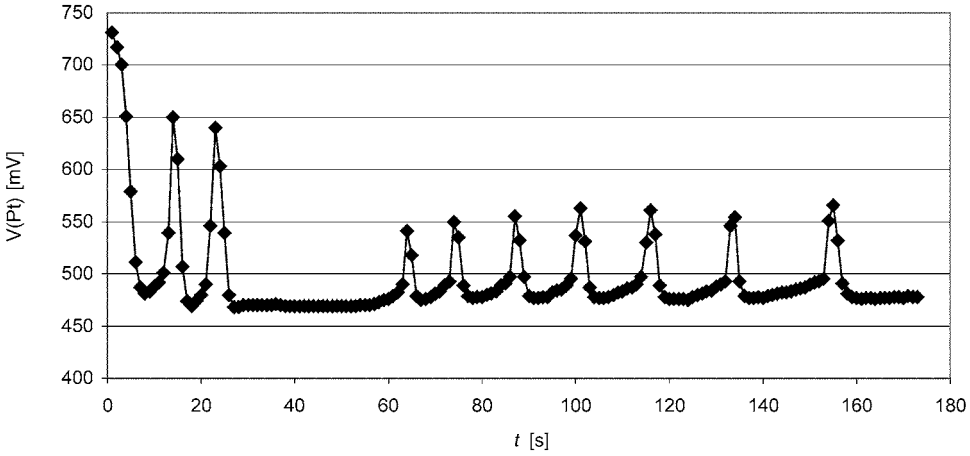

Fig. 2. *The oscillating* Briggs–Rauscher *reaction perturbed by ascorbic acid* (9.16 mM). The inhibition time is the time required for the system to resume oscillation after addition of ASC (added after the third oscillation).

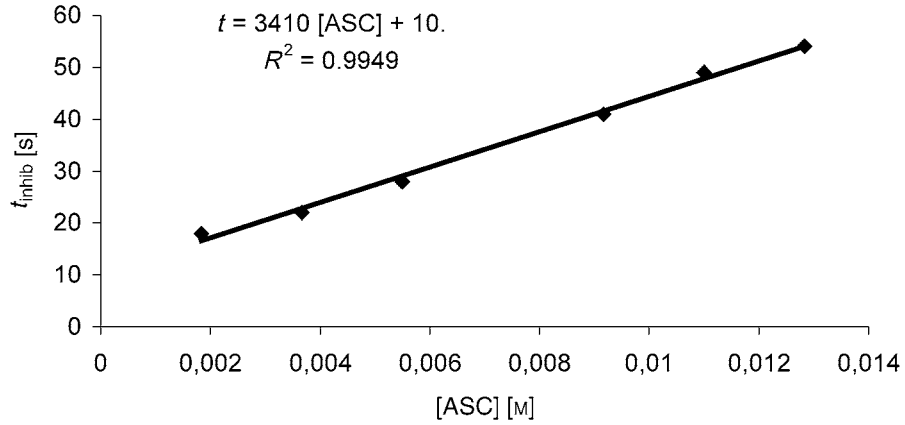

Fig. 3. *Plot of inhibition time* vs. *concentration of ascorbic acid* (ASC) *in a* Briggs–Rauscher *mixture*

generated, which then may inhibit oscillations. To test experimentally whether ASC has the same effect as addition of 1 equiv. of I^-, we added KI solution to the active oscillating system in the same manner as adding ASC. Addition of KI at a final concentration of 9.16 mM caused an inhibition time of *ca.* 100 s (*Fig. 4*). In general, the more KI we added, the longer the inhibition time was, as can be seen from *Fig. 5*.

2.3. *Nonoscillating Reaction of Ascorbic Acid with Iodine Compounds.* Ascorbic acid reacts with I_2 too rapidly to be analyzed after manual mixing in a spectrophotometer. Even at concentrations of both reactants of *ca.* 4×10^{-5} M, the reaction is complete within *ca.* 10 s. The stoichiometry of the reaction, based on changes in absorbance for I_2 ($\lambda = 462$ nm) and/or formation of I_3^- ($\lambda = 353$ nm), is consistent with oxidation of ASC to dehydroascorbic acid (DHA): $ASC + I_2 \rightarrow DHA + 2\,I^- + 2\,H^+$. The reaction of ASC with iodate under acidic conditions is also fast, but can be

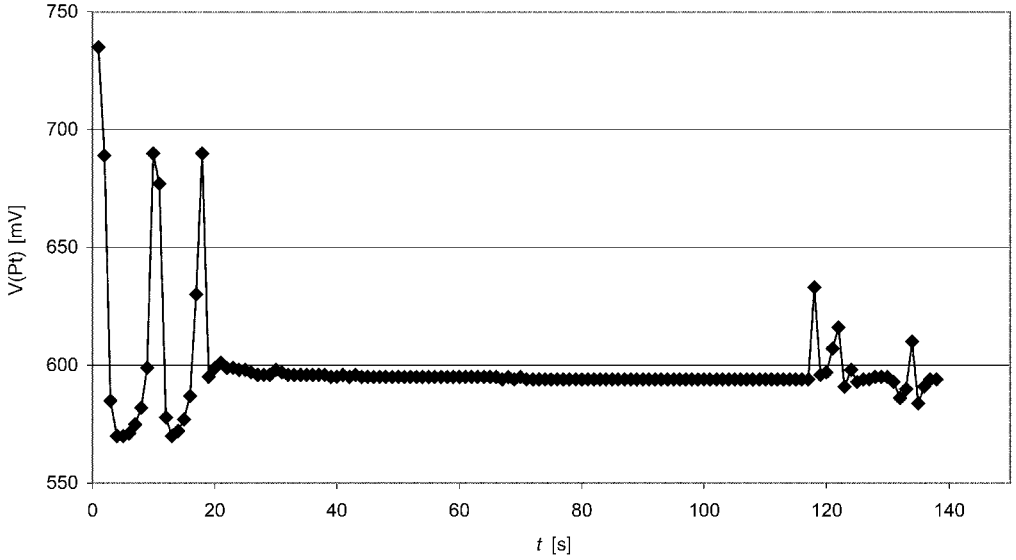

Fig. 4. *The oscillating* Briggs–Rauscher *reaction perturbed by KI* (9.16 mM)

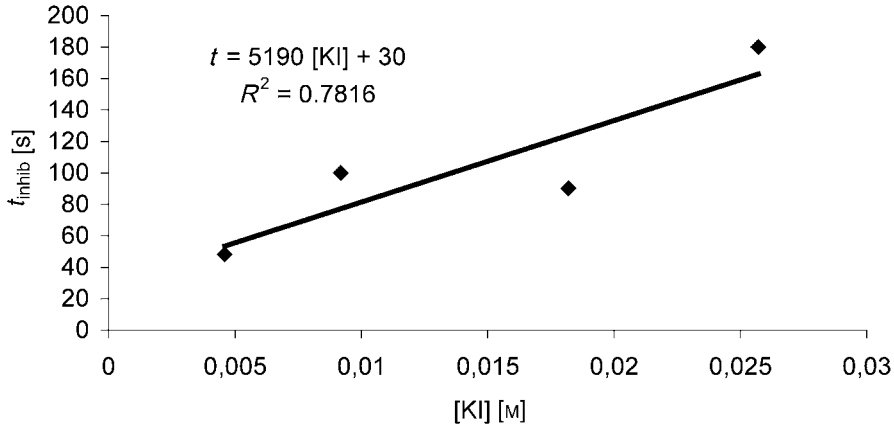

Fig. 5. *Plot of inhibition time* vs. *concentration of KI in a* Briggs–Rauscher *mixture*

observed spectrophotometrically: $2\,H^+ + 2\,IO_3^- + 5\,ASC \rightarrow 5\,DHA + 6\,H_2O + I_2$. Here, I_3^- is a transient intermediate. In summary, the oxidation of ASC by iodate can be modelled by the set of reactions given in *Table 1*.

The time t_{max}, when $[I_3^-]$ reaches a maximum, can be used to determine k_1. The results of several runs are summarized in *Table 2*. The *Gepasi* integration program [8] was used to fit k_1 values, and $k_1 = 1.0 \times 10^4$ M^{-2} s^{-1} was found to be in excellent agreement with both t_{max} and $[I_3^-]_{max}$.

Table 1. *Steps in the Reaction between Iodate and Ascorbic Acid* (ASC). DHA stands for 'dehydroascorbic acid'.

Step	Reaction	Rate or equilibrium constant
1	$H^+ + IO_3^- + ASC \rightarrow DHA + H_2O + HOIO$	k_1 (rate determining)
2	$HOIO + ASC \rightarrow DHA + HOI + H_2O$	k_2 (fast)
3	$HOI + ASC \rightarrow DHA + H^+ + I^- + H_2O$	k_3 (fast)
4	$HOIO + I^- + H^+ \rightarrow 2\,HOI$	$k_4 = 4 \times 10^9$ M^{-1} s^{-1}
5	$2\,H^+ + IO_3^- + I^- \rightarrow HOIO + HOI$	$k_5 = 1430$ M^{-3} s^{-1}
6	$H^+ + I^- + HOI \rightleftharpoons I_2 + H_2O$	$K_6 = 3 \times 10^{12}$ M^{-1}
7	$I_2 + I^- \rightleftharpoons I_3^-$	$K_7 = 710$ M^{-1}

Table 2. *Inhibition of the* Briggs–Rauscher *Reaction by Ascorbic Acid* (ASC) vs. *Acidic Iodate*. The inhibition times t_{max} at maximum antioxidant concentration (I_3^- or ASC), derived both experimentally (exp) and by simulation (calc), are compared.

$[HClO_4]$ [M]	[ASC] [M × 10^4]	$[KIO_3]$ [M × 10^4]	t_{max} (exp) [s]	$[I_3^-]_{max}$ (exp) [M × 10^6]	t_{max} (calc) [s]	$[I_3^-]_{max}$ (calc) [M × 10^6]
0.0266	2.0	2.0	62	0.42	55	0.45
0.0266	2.0	4.0	25	0.68	25	0.46
0.0266	4.0	4.0	25	1.9	20	1.8
0.0532	4.0	4.0	< 10	> 1.9	10	1.8
0.0532	2.0	2.0	22	0.47	20	0.45
0.0160	4.0	4.0	40	1.9	45	1.8
0.0266	4.0	2.0	65	1.8	65	1.8

3. Discussion. – The *FCA* model [7] of the *BR* oscillating system includes, in addition to the seven steps reported in *Table 1*, the following reactions[1]):

$$IO_2^{\bullet} + IO_2^{\bullet} + (H_2O) \rightarrow H^+ + IO_3^- + HOIO \qquad (I5R)$$

$$IO_2^{\bullet} + H_2O_2 \rightarrow HOIO + HOO^{\bullet} \qquad (D2)$$

$$MA \rightleftharpoons EMA \qquad (C1)$$

$$EMA + I_2 \rightarrow H^+ + I^- + IMA \qquad (C2)$$

$$HOIO + (Mn^{2+}) + (H_2O_2) + (H_2O_2) \rightarrow HOO^{\bullet} + HOO^{\bullet} + HOI + (Mn^{2+}) + (H_2O) \qquad (M)$$

$$HOO^{\bullet} + HOO^{\bullet} \rightarrow H_2O_2 + O_2 \qquad (O2)$$

$$H^+ + IO_3^- + HOO^{\bullet} \rightarrow IO_2^{\bullet} + O_2 + (H_2O) \qquad (D4)$$

$$H_2O_2 + HOI \rightarrow H^+ + I^- + O_2 + (H_2O) \qquad (D1)$$

[1]) Abbreviations: MA, malonic acid; EMA, enolic form of MA; IMA, mono-iodinated MA.

Taking into account all the steps above, we were able to simulate a cessation of oscillation for a certain range of ASC concentrations due to the rapid formation of I^-. The agreement was not quantitative, but the trend verified that when $[I^-]$ is too high, oscillations cannot occur.

As expected from the stoichiometry of the reaction, I^- is more effective than ASC at the same concentration (when IO_3^- is reduced to I^- by ASC, a ratio of [ASC]/$[I^-]$ of 3:1 is found). From *Figs. 3* and *5*, one can see that the exerimental inhibition time (t_{inhib}) of the *BR* reaction for 10 mM KI as the antioxidant is *ca.* 2.5 times that of ASC at the same concentration. Our simulations produced similar results. The inhibition time for KI was predicted to be 1.5 to 3 times as high as those for ASC, values approaching the experimentally verified ones at higher antioxidant concentrations.

Ascorbic acid is an important antioxidant in living systems. However, to have a significant inhibitory effect on the *BR* oscillating system, substantially higher concentrations are required compared to, *e.g.*, phenolic additives. We have previously shown that phenolic antioxidants interact with HOO• radicals in the *BR* system. ASC has been shown to mainly interact with iodine in the oxidation states V, III, I, and 0, and, upon reduction to iodide, −I. Reaction of ascorbic acid with HOO• was not included in these simulations. Although the *BR* system can be calibrated to test for ASC (as in *Fig. 5*), inhibition times for addition of ASC to *BR* mixtures should not be compared with those obtained with phenolic substances because of the very different mechanisms of interaction with the oscillating system.

Experimental Part

General. Malonic acid (MA; >99%), $MnSO_4 \cdot H_2O$ (>99%), $NaIO_3$ (>99.5%), L-ascorbic acid (>99%), and KI (>99.5%) were purchased from *Merck* and used without further purification. $HClO_4$, H_2O_2, and other chemicals were of anal. grade. All stock solns. were prepared from doubly distilled, deionized H_2O. $HClO_4$ was standardized by titrations *vs.* 0.1M NaOH standard soln. (*Merck*), and H_2O_2 was standardized daily by manganometric analysis.

Experimental Technique. Oscillations in the *BR* mixtures were followed potentiometrically by recording the electromotive force of a combined redox electrode (*Mettler Toledo InLab 501*). The electrode was 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°. All solns. and reaction mixtures were maintained at a const. temp. (25.0°) by means of a thermostat (±0.1°). *BR* Mixtures were prepared by dispensing the appropriate amounts of reagent stock solns. with pipets or burets and mixing in a 100-ml beaker (total volume: 30 ml). The order of addition was: MA, $MnSO_4$, $HClO_4$, $NaIO_3$, H_2O_2. Oscillations generally start after the addition of H_2O_2. The initial composition of the *BR* mixture was: $[H_2O_2] = 1.20$ M, $[HClO_4] = 26.6$ mM, $[IO_3^-] = 66.7$ mM, $[MA] = 50$ mM, $[Mn^{2+}] = 6.67$ mM. MA or KI (1 ml) were added to 30 ml of an active, well-stirred *BR* mixture after the third oscillation. Absorbance measurements were made on an *HP 8451A* spectrophotometer, with the cell compartment thermostated at 25.0 ± 0.1°. Suitable amounts of reacting solns. were mixed directly in a quartz cuvette of 1-cm path length.

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