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STUDY ON THE INHIBITION OF BRIGGS-RAUSCHER OSCILLATING REACTION

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ABSTRACT: We present the results concerning our exploratory study on the effect of four compounds (ascorbic acid, tartaric acid, pyrogallol and salicylic acid) on the Briggs-Rauscher oscillating reaction. In case of ascorbic acid, tartaric acid and pyrogallol, a linear increase of the inhibition time with the concentration of these substances was observed. We also paid attention on the changes of color and redox potential of the reacting mixture in the moment of addition of the above mentioned compounds. The observations showed that ascorbic acid and tartaric acid manifest an inhibitory effect through their strong reducing properties. Pyrogallol acts like other polyphenolic antioxidants studied before. Salicylic acid, unlike all of the phenolic and polyphenolic compounds described before, did not stop instantly the oscillations.

Keywords: Briggs-Rauscher oscillating reaction, inhibitory effect, ascorbic acid, tartaric acid, pyrogallol, salicylic acid

INTRODUCTION

The Briggs-Rauscher (BR) reaction is one of the most spectacular oscillating reactions, which is a hybrid of the well-known Belousov-Zhabotinskii and the Bray-Liebhafsky reactions. The net chemical transformation of the BR reaction is the oxidation and iodination of malonic acid by hydrogen-peroxide and iodate ion, catalyzed by manganous ion in acidic media [1]. The concentration of intermediates (I_2, I^-, HOI, HIO_2, IO_2•, HOO•, etc.) presents more than one extreme point (i.e.: maximum and minimum) in time. The first mechanistic investigations were carried out by Cooke [2], Noyes and Furrow [3-5], De Kepper and Epstein [6], respectively. As a result of these studies, 30 elementary steps have been identified. Two almost identical skeleton mechanisms were proposed, which described qualitatively well the nonlinear behavior of the reacting system in a batch reactor [5] and in a continuous-flow stirred tank reactor (CSTR) [6]. However there was a discrepancy between the experimental

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and the simulated quantitative features (period time, concentration ranges for different intermediates and the cross-shaped phase diagram) of the reaction [5, 6]. Furrow has recognized that the hydroperoxyl radical mediates the autocatalytic HIO_2 production and plays an important role in the reaction [7]. The modification of the skeleton mechanism by inclusion of the elementary steps involving IO_2• and HOO• improved the predicting ability of the model [7, 8].

Cervellati *et al*. reported that the addition of mono- and polyphenolic compounds to the active mixture causes a temporary but instant cessation of oscillations [9, 10]. The time elapsed between the cessation and the subsequent regeneration of the oscillatory regime was denominated as inhibition time. A linear correlation was found between the concentration and the inhibition time for every phenolic substance added in the BR-mixture. The inhibitory effect was accounted for a fast reaction involving the phenolic compound and HOO• radical. Since polyphenols are known to be effective free radical scavengers, they reduce drastically the concentration of HOO• in the BR-mixture. As soon as the antioxidant is totally consumed, the HOO• concentration rises up to the critical range, where the oscillations reappear. The side-reaction between the phenolic compounds and the oxidative species of the BR mixture was also taken into account [10, 11]. For quantitative modeling of the inhibitory effect, the modified skeleton model [8] was complemented with the steps involving the antioxidant [11]. Based on these results it was possible to develop a new analytical method for determination of the antioxidant activity of free radical scavengers.

In contrast with the other antioxidant assays (Trolox Equivalent Antioxidant Capacity /TEAC/, Total Radical Trapping Parameter /TRAP/, Ferric Ion Reducing Antioxidant Parameter /FRAP/, and Oxygen Radical Absorbance Capacity /ORAC/), in the method proposed by Cervellati *et al*. the antioxidants react with HOO• radical [12]. This radical is a reactive oxygen metabolite produced in the human body, to which oxidative stress, cell damage, aging, cancer and other diseases are associated [13, 14]. Therefore the method validated by Cervellati *et al*. gives more reliable data with respect to the reactivity of the antioxidants with reactive oxygen species (ROS).

Furrow *et al*. found that the better known antioxidant, ascorbic acid acts different than the polyphenols. Being a strong reducing agent, the ascorbic acid is oxidized instantly by the reactive oxy-iodide species when is added to the BR-mixture, while iodide ion is produced. An inhibitory effect of iodide ion on the BR-reaction was observed. The qualitative mechanistic interpretation of inhibitory effect of iodide ion was also given [15]. Since the ascorbic acid added to the BR-mixture does not react with HOO• radical, it was concluded that the BR-method is not suitable to determine the antioxidant activity of ascorbic acid.

RESULTS AND DISCUSSION

The effect of ascorbic acid on the BR-reaction

Foremost we tried to reproduce the experiments made by Furrow *et al*. [15]. When we added ascorbic acid solution, the color of the oscillating BR-mixture changed suddenly to brown. At the same time the redox potential rise (ΔE) instantly and then decreased gradually during the inhibition time (Fig.1). When starch was also present in the BR mixture, a sudden deep blue coloration was noticed after addition of the ascorbic acid solution. The blue color faded out gradually during the inhibition period. Nor the appearance of the brown color, neither the redox potential rise was noticed by Furrow *et al.* in the moment of ascorbic acid solution to the BR-mixture.

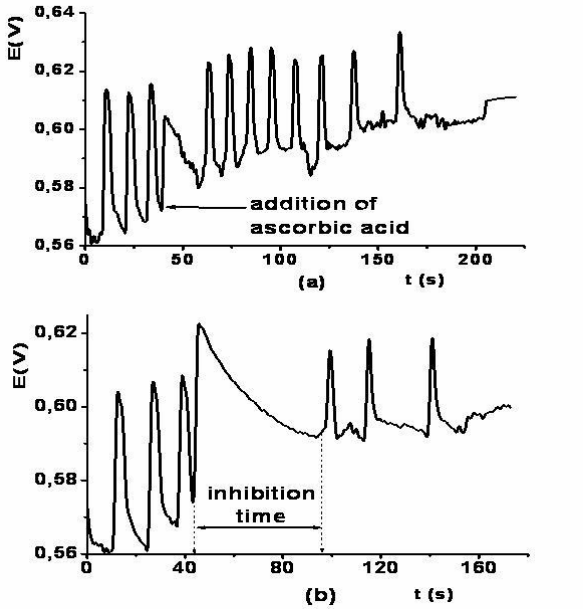


Figure 1. Effect of 3,36 mM (a) and 7,70 mM (b) ascorbic acid on the BR-reaction.

Our observation suggests that there is a rapid and significant I_3^- production when ascorbic acid is added to the BR-mixture. We attribute the sudden change of the redox potential to the fast and coupled redox reaction steps involving ascorbic acid and the oxidizing iodide species (IO_3^-, HOI, HIO_2 and I_2) of the BR-mixture. However the reaction has been monitored using a bright platinum electrode, which is reversible for every redox couple existing in the solution, the measured potential is a mixed potential (i.e. every redox system contributes to the measured potential). Therefore it is not possible to assign directly the potential change to any change of concentration of the species.

The reproducibility of the inhibition time was good. The inhibition time and the magnitude of the redox potential change (ΔE) varies linearly with the ascorbic acid concentration within the 3,36−9,97 mM interval (Figure 2.).

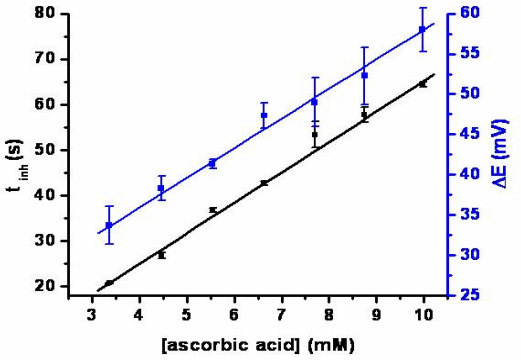


Figure 2. Plot of the inhibition time and the redox potential change against the concentration of ascorbic acid.

$$t_{inh} = (6{,}7\pm0{,}1)\cdot[ASC] - (1{,}8\pm0{,}5) \quad R^2=0{,}9985 \quad (1)$$
$$\Delta E = (3{,}7\pm0{,}3)\cdot[ASC] - (21{,}2\pm1{,}5) \quad R^2=0{,}9770 \quad (2)$$

If the ascorbic acid concentration was higher than 9,97 mM, the oscillations did not restart after a long time (more than 350 s), however the color of the mixture remained brown or blue (depending on the absence or presence of starch in the initial BR-mixture).

The features of restarted oscillations (amplitude, period) were different of those observed in the non-inhibited reactions. The amplitude of the restarted oscillations were smaller, the period length was longer and steadily increased in the successive periods. These latter observations are in accordance with those reported by Furrow *et al.* [15].

The effect of tartaric acid on the BR-reaction

Since Cervellati *et al*. has shown that the hydroxyl groups are responsible for the antioxidant effect of mono- and polyphenols [9-11], we tried to find out whether a compound containing aliphatic hydroxyl group can inhibit the BR-reaction. Also the ascorbic acid contains two aliphatic hydroxyl groups, which are not affected by the oxidation. However it would be difficult to figure out whether these hydroxyl groups have any inhibitory effect [15]. Thus, we choose tartaric acid, a simple, bi-functional compound to our study. On the other hand this compound can be found in white wines as *trans*-caffeoyl-tartaric acid. Most of the antioxidant activity of the Riesling white wine was accounted for this ester and hydroxy-cinnamic acid derivatives [16]. However in the human stomach the *trans*-caffeoil-tartaric acid is hydrolysed by the gastric juice to caffeic acid and tartaric acid. The antioxidant activity

of caffeic acid is already known from the literature [10], but there is no information about the tartaric acid. Tartaric acid contains, like ascorbic acid, two aliphatic hydroxyl groups, and it is a reducing agent at the same time.

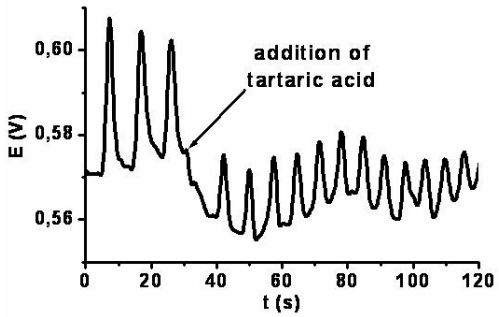


Figure 3. The effect of 10,2 mM tartaric acid on the BR-reaction.

We observed a drop of redox potential (ΔE) in the moment of addition of tartaric acid, while the mixture remains colorless. The rapid change of redox potential suggests that a fast redox reaction involving tartaric acid and oxy-iodine species occurred. However compared to the experiments carried out with ascorbic acid, the main difference is that I_3^- was not observed. The more likely is that I^-, HOI or HIO_2 colorless oxy-iodine species were formed. If the concentration of these compounds exceeds a certain limit, they also perturb the oscillatory regime for a while [4, 5, 6, 15].

The inhibition time increased linearly with the concentration of the tartaric acid only within the range of 9,1-17,1 mM (Eq.3). At concentrations larger than 17,1 mM the oscillations did not restart at all.

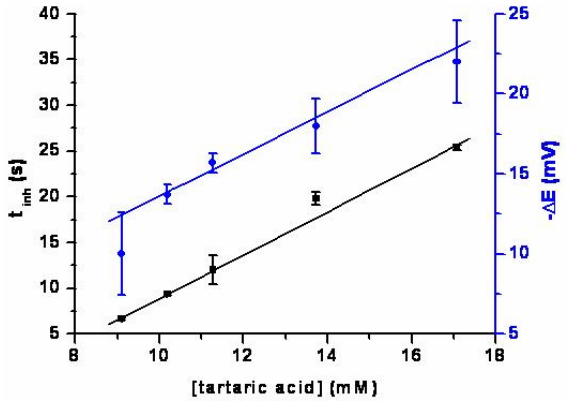


Figure 4. Plot of the inhibition time and the redox potential change against the concentration of tartaric acid.

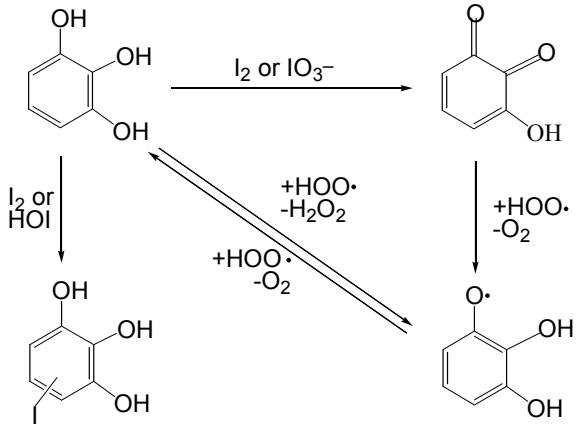
t_{inh} = (2,4±0,1)·[tartaric acid] − (14,8±0,7) R^2=0,9972 (3)

$-\Delta E$ = (1,3±0,3)·[tartaric acid] − (0,4±2,5) R^2=0,9180 (4)

The effect of pyrogallol on the BR-reaction

The third compound we studied was pyrogallol. This polyphenol can be prepared by decarboxylation of gallic acid, a naturally occurring organic acid which has an antioxidant activity. Addition of this pyrogallol to the oscillating mixture caused brown coloration and an inhibition time. No significant redox potential change has been noticed in the moment of addition of pyrogallol solution to the mixture, or during the inhibition period. The brown color faded out gradually during the inhibiton period. When starch was also present in the initial BR-mixture, no blue coloration was noted after addition of pyrogallol and/or during the inhibition period. Similarly to other polyphenolic compounds, pyrogallol appeared to be an effective inhibitor of the BR-reaction: it caused an inhibition time of 133,5 s at a concentration of 33 μM. The periods of the oscillations after the inhibition time were the same as those of non-inhibited reaction.

The pyrogallol is also a very strong reducing agent. Therefore the strong oxidants present in the BR reaction oxidized it instantly. However in this case the appearance of the brown color cannot be assigned to I_3^- production because of the following facts: pyrogallol was added to (i) acidic iodate, and (ii) acidic iodate and hydrogen peroxide mixture. In both cases a brown coloration has been observed.



Scheme 1

This coloration did not disappear when tiosulphate solution has been added to the mixture, suggesting that the colored product was not I_3^-, the more likely an oxidized form of pyrogallol. On the other hand based on the stoechiometry, the production of I_3^- in large quantities can be excluded (i.e. the pyrogallol was added to the BR-mixture in the concentration range of several 10 μM which upon oxidation by the oxy-iodine species does not produce such large quantity of I_3^- which would cause a visible and durable coloration).

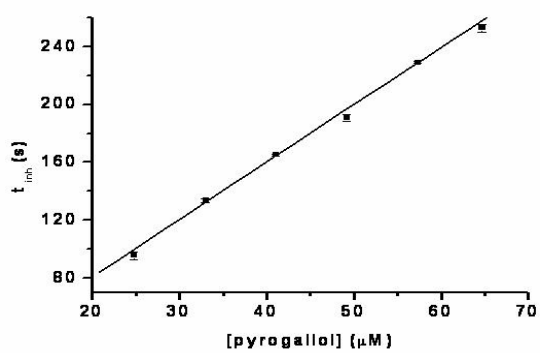


Figure 5. Plot of the inhibition time against the concentration of pyrogallol.

When pyrogallol is oxidized, the aromatic electronic structure is braked up. The product is an unsaturated cyclic di-oxo-compound with a planar structure. The hydroxyl groups in the 1 and 3 positions can not be oxidized, because after the breaking up of the aromatic ring it is not possible to reach a stable electronic structure, hence *meta*-quinones does not exist. Based on the same consideration, the simultaneous oxidation of each hydroxyl group is also impossible [11].

$$t_{inh} = (3{,}96\pm0{,}07)\cdot[\text{pyrogallol}] + (1{,}7\pm3{,}9) \quad R^2=0{,}9986 \qquad (5)$$

Cervellati *et al.* pointed out that the quinone derivatives also have antioxidant activity [11]. The inhibitory effect of quinone derivatives is very complicated, and it is not yet fully unraveled. Based on thermodynamic considerations, the HOO· can reduce the quinone derivatives back to polyphenolic compounds. The inhibition time depends on the relative rates of the possible consecutive-parallel steps shown in Scheme 1.

The effect of salicylic acid on the BR-reaction

It was shown that monophenolic compounds (i.e.: 3-(4-hydroxy-3-metoxyphenyl)-prop-2-enoic acid and 4-hydroxy-3-metoxy-benzeneacetic acid) are as efficient inhibitors of the BR reaction as polyphenols [10]. We tried to inhibit the BR reaction with salicylic acid a very simple, naturally occurring monophenolic compound. In the few ten-fold μM concentration range this compound has no effect on the reaction. When we added in the BR mixture in the mM concentration range, we observed that the oscillations were not stopped at all, even at higher concentration they were not stopped immediately. However the amplitudes were progressively reduced in the successive periods. The period length was not affected by the presence of salicylic acid in concentrations lower than 6,18 mM. At 6,18 and 6,94 mM salicylic acid concentration the amplitude reduction and period lengthening was significant. At the last concentration the oscillations were completely stopped for 120 seconds, but only after a damping period of 140 seconds (Figure 6 and Table 1).

The shape of oscillations was modified in the presence of salicylic acid. The uninhibited oscillations have typically four segments [17]: a slightly decreasing segment when the iodine intermediate is consumed relatively slowly by the organic substrate and iodide ion is produced. Then a fast increase in redox potential happens because of the rapid radical steps which consume iodide ions. The slowly decreasing segment corresponds to the slow radical steps that produce iodide ions. During the fast decreasing step, iodine is produced and the color of the mixture changes into brown and then the cycle repeats. However after addition of salicylic acid the oscillations have only two segments: an increasing and a decreasing one. It is also notable that after the addition of salicylic acid the mixture remained transparent until the end of the reaction even in presence of starch, and the potential range of the oscillations decreased slightly from 745-765 to 720-740 mV.

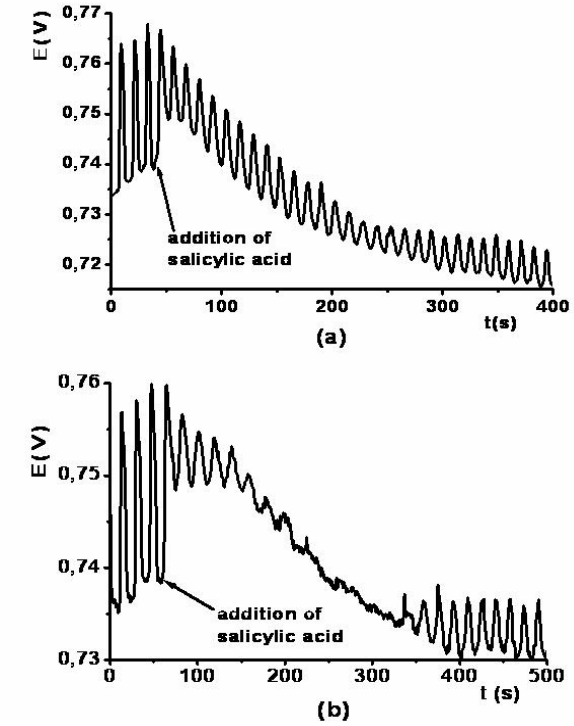


Figure 6. Effect of 5,41 mM (a) and 6,94 mM (b) salicylic acid on the BR reaction.

The unusual effect of salicylic acid constitutes a subject of further studies. By our knowledge, in the literature there is no description of this kind of damping effect of an added substance upon the BR reaction.

We found two descriptions about “spontaneous” oscillation damping phenomena (i.e. without adding a substance in the mixture) in the literature: Wittmann *et al*. used in the Belousov-Zhabotinsky reaction instead of malonic acid an oxalic acid - acetone mixture as organic substrate. They observed that after the first regular periods, the amplitudes of the following oscillations were progressively reduced. In some cases the oscillations were completely damped, and after some time they reappeared. The shape and period length of oscillations observed before and after the oscillation break were different [18].

Table 1. The effect of salicylic acid concentration on the amplitudes and the periods of the oscillations. (a) The salicylic acid solution was added after the minimum of the 3rd oscillation. (b) Completely stopped oscillations for 120 s.

Conc.	4,65 mM		5,41 mM		6,18 mM		6,94 mM	
N	Amp (mV)	p (s)	Amp (mV)	p (s)	Amp (mV)	p (s)	Amp (mV)	p (s)
1	30	13,8	29	12,8	30	13,4	27	14,6
2	31	12,6	28	11,6	31	13,4	26	14,8
3	34	13,2	30	12,2	28	15,4	28	14,8
4 [(a)]	23	13,0	18	11,6	16	17,0	11	17,8
5	17	13,0	14	11,6	11	16,4	8	19,2
6	17	13,8	14	12,0	10	18,4	6	17,6
7	18	14,0	14	12,2	11	19,2	5	20,2
8	19	13,8	14	12,2	9	17,4	5	19,8
9	19	14,0	14	12,0	10	18,4	4	19,4
10	18	13,8	14	12,6	9	17,8	3	20,2
11	20	13,8	13	12,4	11	17,8	3	12,4
12	18	13,8	12	11,6	11	18,4	0 [(b)]	120 [(b)]
13	19	14,0	12	12,6	10	17,8	2	11,8
14	18	14,2	11	12,6	12	17,4	4	15,8
15	18	14,2	9	12,2	10	18,4	7	16,8
16	17	14,6	10	12,4	9	18,4	5	17,6
17	17	13,8	8	12,4	10	17,8	6	16,4
18	14	14,8	7	12,8	7	17,4	6	16,4
19	14	14,2	5	13,8	8	18,4	5	15,6
20	14	14,6	5	11,6	7	18,0	7	15,6

Szabó studied the effect of iodate ion concentration upon the BR reaction. In BR mixtures containing 20 mM, 17,5 mM, and 15 mM iodate ion respectively; he observed a break after the first large amplitude oscillation. At 1,25 mM iodate ion concentration the damping of oscillations occurred only after the 6th period. In this case the duration of oscillation break was 50 seconds [19]. In any cases the amplitudes, the period lengths and the shape of oscillations before and after the break were different.

However it is very important to emphasize that the oscillation breaks observed by Wittmann *et al.* and Szabó should be not considered as inhibition time, because these were not caused by an added substance.

CONCLUSIONS

Among the numerous methods developed to determine the antioxidant activity, the method based on the Briggs-Rauscher oscillating reaction has a unique advantageous feature: the antioxidant reacts with hydroperoxyl radical. This radical is a reactive oxygen species which is also produced by the human body during the metabolism. Therefore the hydroperoxyl radical can be considered to be a biologically more relevant substrate, than the various organic substrates of the other methods. However the BR-method has also its own limitations due to the fact that there are strong oxidizing agents which can oxidize the antioxidant added to the mixture. Furrow *et al.* has shown that ascorbic acid is readily oxidized by these oxidizing agents, while iodide ion is produced which causes a break in the oscillations.

Our aim was to reproduce the effect of ascorbic acid on the BR-reaction and to explore the applicability of this method in case of three other compounds: tartaric acid, pyrogallol and salicylic acid. We have shown that in case of the first three compounds there is a linear relationship between the concentration and the inhibition time. One would be tempted to calculate the relative antioxidant activities based on the inhibition times, but we have shown that all these compounds act differently on the reaction; therefore such a comparison is useless. The fourth compound had the most surprising behavior: we observed a damping effect on the oscillations.

EXPERIMENTAL SECTION

Three stock solutions have been prepared with the following composition:

(A). 1,84 M H_2O_2 (Merck, p.a),
(B). 0,27 M KIO_3 (AnalytiCals, p.a), 0,1 M H_2SO_4 (Riedel de Haen, p.a)
(C). 0,2 M $CH_2(COOH)_2$ (Reachim, p.a), 0,026 M $MnSO_4$ (Reactivul, p.a).

These solutions were thermostated to 25±0,1ºC . A volume of 10,0 of A, 5,0 ml of B and 5,0 ml of C stock solution has been mixed in a double-walled glass reactor, which was thermostated to 25±0,1ºC. The composition of a reactive system was: $[CH_2(COOH)_2]_0=5\cdot10^{-2}$ M, $[H_2O_2]_0=0,92$ M, $[H_2SO_4]_0=2,5\cdot10^{-2}$ M, $[MnSO_4]_0=6,5\cdot10^{-3}$ M, $[KIO_3]_0=6,75\cdot10^{-2}$ M. A Falc-type magnetic stirrer ensured the forceful stirring of the reacting mixture. The solutions of studied compounds [ascorbic acid (Reanal, p.a), tartaric acid (2,3-dihydroxy-succinic acid, used as neutral sodium salt, Shering Kahlbaum, p.a), pyrogallol (1,2,3-trihydroxy-benzene, Laboratorium Chemiczne Gallol, p.a), salicylic acid (2-hydroxy-benzoic acid,

used as sodium salt, Reactivul, p.a)] were added with a micropipette (Labsystems Finnpipette) every time after the minimum of the third oscillation. In some cases we added 1,0 ml 1,0 w/w% starch (Reactivul, p.a) solution in the initial BR-mixture in order to visualize the variation of concentration of iodine, one of the intermediates of the reaction. Every measurement was performed at least three times. The reaction was monitored potentiometrically, employing a bright platinum and double-junction saturated calomel electrode. The potential difference between these electrodes was registered by a computer equipped with National Instruments® Data Acquisition Card. The sampling frequency of data acquisition was set to 5 s^{-1}. For data processing Origin® 6.0 program was used. The average and the standard deviation of the inhibition times were calculated. Inhibition times were plotted against the concentration of compounds, the equation of the straight lines was determined using a weighted linear regression [20].

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