Inhibitory Effects by Antioxidants on the Oscillations of the *Briggs-Rauscher* Reaction in Mixed EtOH/H_2O Medium

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The *Briggs-Rauscher* (*BR*) oscillating reaction in mixed 20% EtOH/H_2O (*v*/*v*) medium is studied together with the inhibitory effects by the addition of hydroalcoholic solutions of antioxidants on its oscillatory regime. As in aqueous *BR* mixtures, the inhibitory effect consists of an immediate cessation of oscillations, an inhibitory time that linearly depends on the concentration of the antioxidant added, and subsequent regeneration of oscillations. The effects of several water-insoluble and water-soluble antioxidants were investigated: at a parity of concentration of antioxidant added, inhibition times in the mixed EtOH/H_2O medium are 2–3 times lower than those reported previously in aqueous solution. However, the mechanism of the *BR* reaction, as far as that of the inhibitory effect, seems to be the same in either aqueous or mixed medium. The findings reported and discussed here are an indication that the analytical procedure to assess the activity of free radical scavengers based on the *BR* reaction can be extended to lipophilic antioxidants.

1. Introduction. – This paper is the third of a series [1][2] concerning the inhibitory effects by antioxidants on the oscillations of the *Briggs-Rauscher* (*BR*) reaction [3], which consists of the iodination and oxidation of an organic substrate (in general, malonic acid (MA) or its derivatives) by acidic iodate in the presence of H_2O_2 and with the Mn^{2+} ion as catalyst. The inhibitory effect consists of an immediate cessation of oscillations, an inhibition time that linearly depends on the concentration of the antioxidant added, and subsequent regeneration of oscillations.

Among the main intermediates for which concentrations oscillate in the *BR* mixture, there is the hydroperoxyl radical HOO$^{\bullet}$. It was well-established that phenolic OH group(s) attached to ring structures present in several components of plants as well as in synthetic compounds are responsible for the antioxidant activity [4] against free radicals of oxygen. We have ascribed inhibitory effects on the oscillations of the *BR* reaction to scavenging of HOO$^{\bullet}$ radicals by phenolic OH contained in the antioxidants added to an active *BR* mixture [1][2]. The dependence of the inhibition time (*i.e.*, the time elapsed between the cessation and the regeneration of the oscillatory regime) on the concentration of antioxidants added was found to be linear over a wide range of concentration [2].

On the basis of inhibition times, it was possible to set up a new method (the *BR*-reaction method) to assess the relative antioxidant activity with respect to a substance chosen as a standard [2]. This new method has been successfully tested on 16 German

white wines [5]. It was found that inhibition times correlate well with the total phenolic content of the wines examined. The order of the antioxidant activities of the wines was equal in some cases with the order of the TEAC (Trolox equivalent antioxidant capacity [6]) values but differed for some wines.

Until now, the reported *BR*-reaction method [2] is suitable only for H_2O-soluble antioxidants. It works at pH $\approx$ 2, similar to that of the fluids in the human stomach.

The main goal of the present work is to try to improve the method in order to make it suitable for lipophilic antioxidants. In fact, preliminary experiments showed that the *BR* reaction can also occur in mixed EtOH/H_2O medium. In a 20% EtOH/H_2O (*v*/*v*) mixture, antioxidants insoluble in H_2O, such as caffeic acid, ferulic acid, and isoferulic acid, are soluble at suitable concentrations to observe inhibitory effects on the oscillations of the *BR* reaction in hydroalcoholic medium. We decided to study the behavior of the oscillating *BR* reaction in this EtOH/H_2O medium, and the inhibitory effects of a number of H_2O-insoluble and H_2O-soluble antioxidants. We include the latter to compare the results with those previously reported for the aqueous medium [2]. Here, we report and discuss the results obtained in the mixed EtOH/H_2O medium. We will also discuss the possibility of implementing the *BR*-reaction method with organic solvents other than EtOH.

2. Results. – 2.1. *Comparison of* BR *Oscillations in Aqueous and Mixed 20% EtOH/H_2O (v/v) Medium*. The recordings of the oscillations of the bright-platinum potential in aqueous and in mixed 20% EtOH/H_2O (*v*/*v*) at the same initial concentrations used in [2] are reported in *Fig. 1*, *a* and *b*, respectively.

As can be seen, the numbers of oscillations and the oscillatory time (*i.e.*, the duration of the oscillatory regime) are much smaller in the mixed medium than in

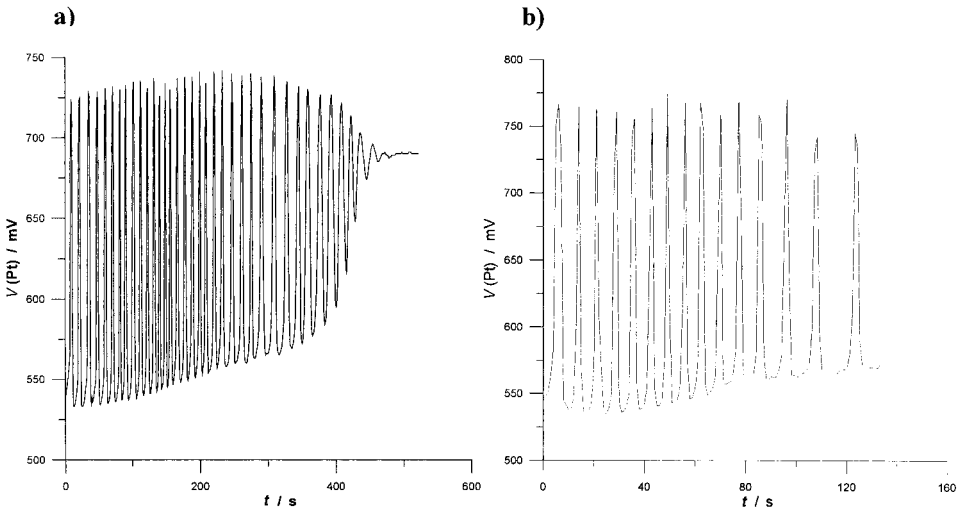

Fig. 1. a) *Recording of the oscillations of the bright-Pt potential in aqueous solution.* Initial conditions: $[H_2O_2] =$ 1.20M, $[HClO_4] = 0.0266$M, $[IO_3^-] = 0.0667$M, $[MA] = 0.050$M, $[Mn^{2+}] = 0.00667$M. b) *Recording of the oscillations of the bright-Pt potential in mixed 20% EtOH/H_2O* (*v*/*v*) *at the same initial conditions.*

aqueous solution. Previous work [7][8] reported that decreasing $[IO_3^-]_o$ increases the number of oscillations and the oscillatory time but, at the same time, the amplitude of oscillations decreases. To find a compromise between these parameters, we studied the effect of decreasing initial $[IO_3^-]$ on them, maintaining the initial concentrations of other reagents and catalyst constant in the mixed medium. The results are reported in *Table 1*. *Fig. 2* shows the behavior of oscillatory time and oscillations amplitude *vs.* time.

Table 1. *Variation of Oscillation Parameters in Mixed EtOH/H_2O Medium with Initial $[IO_3^-]$*

$[IO_3^-]_o$ in mixture	Oscillations		
	Duration/s	Number	Amplitude/mV
$6.67 \cdot 10^{-2}$ M	99	12	235
$5.33 \cdot 10^{-2}$ M	132	15	221
$4.00 \cdot 10^{-2}$ M	171	21	208
$3.33 \cdot 10^{-2}$ M	297	35	204
$2.66 \cdot 10^{-2}$ M	335	44	199
$2.00 \cdot 10^{-2}$ M	414	58	190
$1.33 \cdot 10^{-2}$ M	557	75	166
$6.67 \cdot 10^{-2}$ M	413	51	82

We found a good compromise at an initial $[IO_3^-] = 3.33 \cdot 10^{-2}$ M. The behavior of log $[I^-]$ *vs.* time for an aqueous *BR* mixture with $[IO_3^-] = 6.67 \cdot 10^{-2}$ M (as in [2]) and that for a *BR* mixture in the hydroalcoholic medium with $[IO_3^-] = 3.33 \cdot 10^{-2}$ M are reported in *Fig. 3, a* and *b*, respectively.

As can be seen, the number and duration of oscillations are almost the same even though the amplitude is somewhat greater in the case of the aqueous *BR* mixture. In *Fig. 4, a* and *b*, the initial parts of the recordings shown in *Fig. 3*, *a* and *b* are reported.

Although there are slight differences in the oscillation period and in the induction time, the shape of log $[I^-]$ oscillations is the same in the aqueous and hydroalcoholic media. This strongly suggests that the mechanisms proposed for the *BR* reaction in aqueous medium under batch conditions [9][10] are also valid in mixed 20% EtOH/H_2O (*v*/*v*) even though the rate constants of the mechanistic steps are different in the aqueous and the mixed medium.

All halate-driven oscillators exhibit a common mechanistic pattern. Halate is reduced by two separate stoichiometric processes, one of which is radical and the other nonradical [9]. The two processes generate very different steady-state concentrations of halous acid (an important intermediate in these oscillators), and dominance between them is switched by a critical condition that consists of attainment of a specific concentration of halide ion. A third stoichiometric process (the halogenation of the organic substrate) couples with the two other processes to generate the net chemical change that drives the oscillations. In the *BR* [3] IO_3^--driven oscillator, the radical and nonradical reduction processes have identical stoichiometries [9]. When $[I^-]$ is high, the nonradical process dominates (point *A* in *Fig. 4*,*a*); when $[I^-]$ falls under a critical value (point *B* in *Fig. 4*,*a*), the reaction passes under the control of the radical process (line *B* – *C* in *Fig. 4*,*a*). When the $[I^-]$ becomes very low (point C in *Fig. 4*,*a*), the third process intervenes, producing I^- (line C – D in *Fig. 4*,*a*). The production of I^- continues

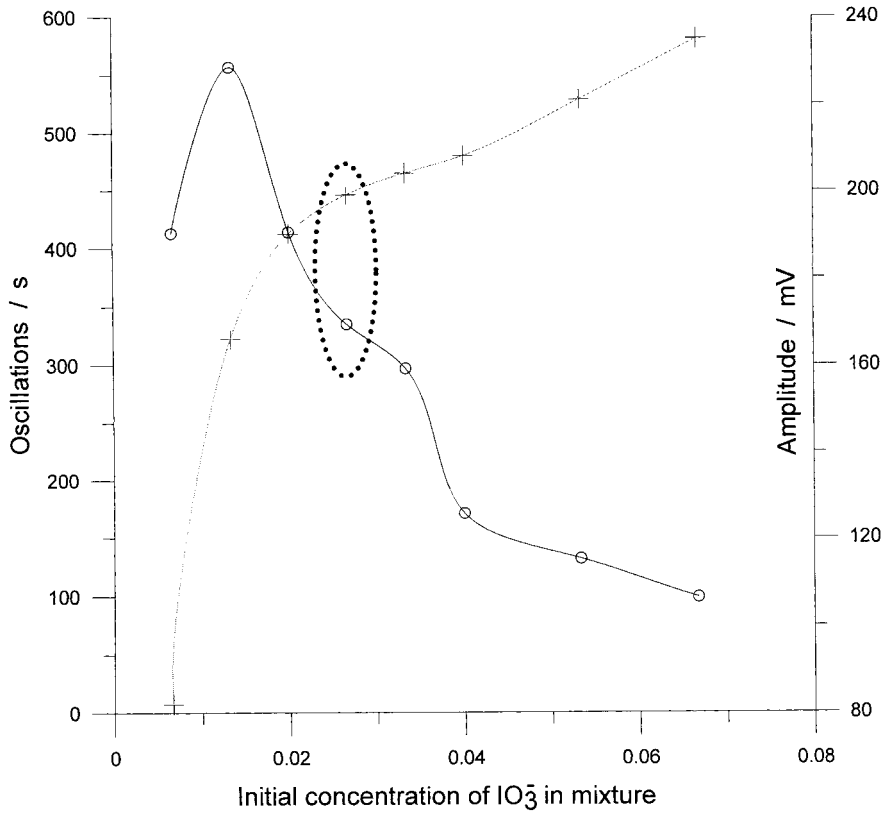

Fig. 2. *Behavior of oscillatory time* (○) *and oscillation amplitude* (+) *with initial concentration of* IO_3^- *in EtOH/* H_2O BR *mixture* (other reagents concentrations as reported in the legend of *Fig. 1*). Circled points show the chosen compromise; 1.0 ml of the hydroalcoholic solution was added after the third oscillation.

until reaching a high concentration that brings the reaction again under the control of the nonradical process. The cycle is then repeated, so producing the oscillations.

2.2. *Effect of Temperature on the Inhibition Time.* In the previous paper [2], we reported the observation that the temperature of an active noninhibited or inhibited *BR* mixture slightly rises in spite of the thermostasis. A study of the variation of inhibition times in a narrow range (2–3°) around 25° showed a linear dependence of inhibitory time on the temperature. These relationships were used to obtain 'corrected' inhibition times at exactly 25.0° [2].

However, in the case of inhibited mixtures, we noted that, during the inhibitory phase, the temperature of the mixture remains practically constant and identical to that of the thermostatic bath. We observed the same behavior in the mixed medium, as shown in *Fig. 5* for a mixture inhibited by caffeic acid.

The same behavior was observed for other antioxidants as ferulic and isoferulic acids, 3,4-dihydroxybenzoic acid (3,4-DHBA), and resorcinol. Therefore, when the mean temperature of the mixture during the inhibitory phase is only slightly different

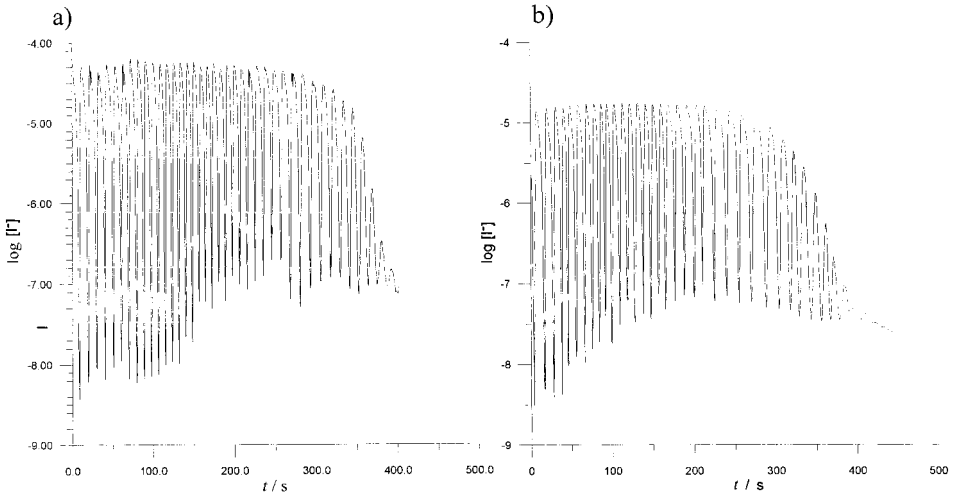

Fig. 3. a) *Recording of log* [I^-] vs. *time for a* BR *aqueous mixture.* Initial conditions as in the legend of *Fig. 1.* b) *Recording of log* [I^-] vs. *time for a* BR *EtOH/H_2O mixture.* Initial conditions: [IO_3^-] = 0.0333M, other concentrations as in the legend of *Fig. 1.*

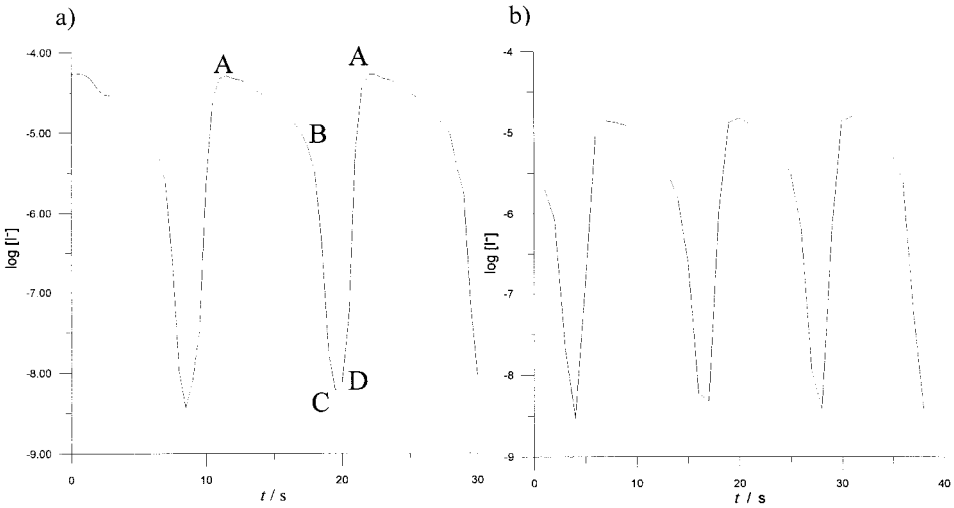

Fig. 4. a) *Initial part of the recording shown in* Fig. 3,a. b) *Initial part of the recording shown in* Fig. 3,b.

from that of the thermostatic bath (kept fixed at $25.0 \pm 0.1^\circ$), the correction is not necessary. In fact, the t_{inhib} corrected at exactly 25.0° differs only by a few seconds from the measured one. This is clearly shown in *Fig. 6* where the behavior of the experimental t_{inhib} at *ca.* 25° and of the corrected t_{inhib} at 25.0° *vs.* the concentration of the antioxidant added are reported for ferulic acid.

From the plot, it is evident that a variation of a few seconds on inhibition times greater than 500 s is practically negligible. In any case, in all experiments we have

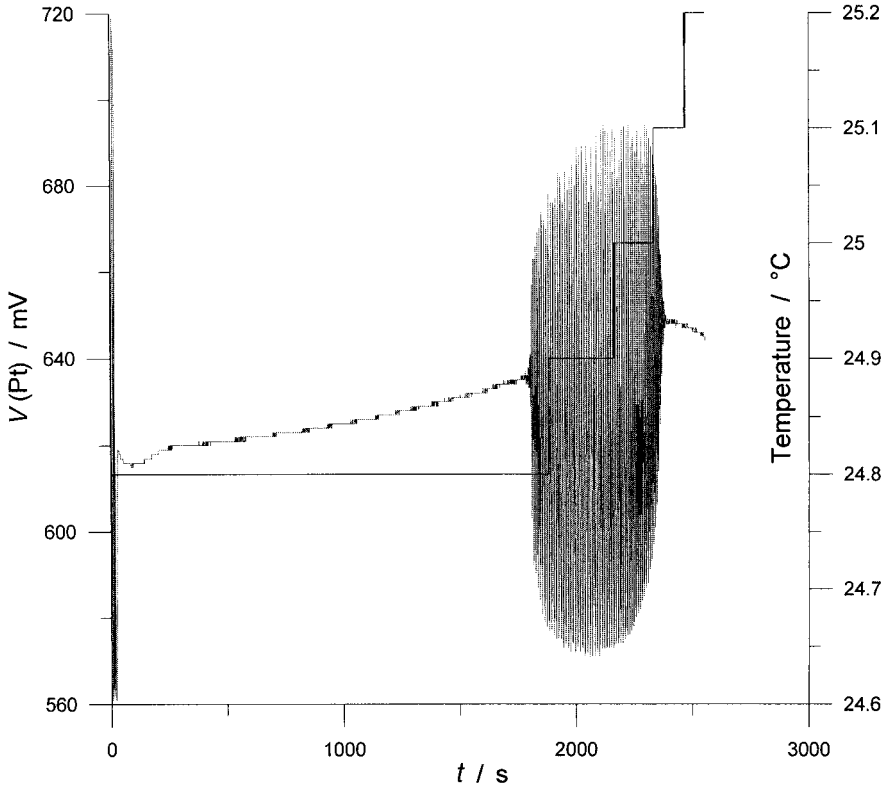

Fig. 5. *Recording of the potential of the bright-Pt electrode and of the temperature* vs. *time when 1.0 ml of a solution of caffeic acid was added to 30 ml of a thermostated* BR *EtOH/H_2O mixture.* Initial concentration of caffeic acid = 8.06 μM. Temperature of the thermostatic bath = 25.0 ± 0.1°. Initial conditions: the same as in *Fig. 3, b.*

controlled the temperature during the inhibitory phase to be sure to make measurements in the very narrow temperature range 24.5–25.5°.

2.3. *Inhibitory Effects of a Series of Antioxidants in Mixed EtOH/H_2O Medium.* Structures of antioxidants considered are reported in *Fig. 7.* For each antioxidant, we studied the dependence of the inhibition time on the concentration. As an example, the graph t_{inhib} *vs.* concentration for isoferulic acid (iFA) is reported in *Fig. 8*[1]).

2.4. *Relative Activity Calculations.* The linear dependencies of t_{inhib} *vs.* concentration for most of the substances studied are shown in *Fig. 9.*

As can be seen, 2,5-DHBA is much less active than all other antioxidants in aqueous solution [2], as well as in mixed EtOH/H_2O medium. Also in the mixed medium, below a certain concentration of antioxidant added (different for each antioxidant), the behavior deviates from linearity. In fact, at low concentrations of antioxidant added, the inhibition times become too low to be measured. There is a threshold under which

[1]) These plots for all the antioxidants studied are available from the authors upon request.

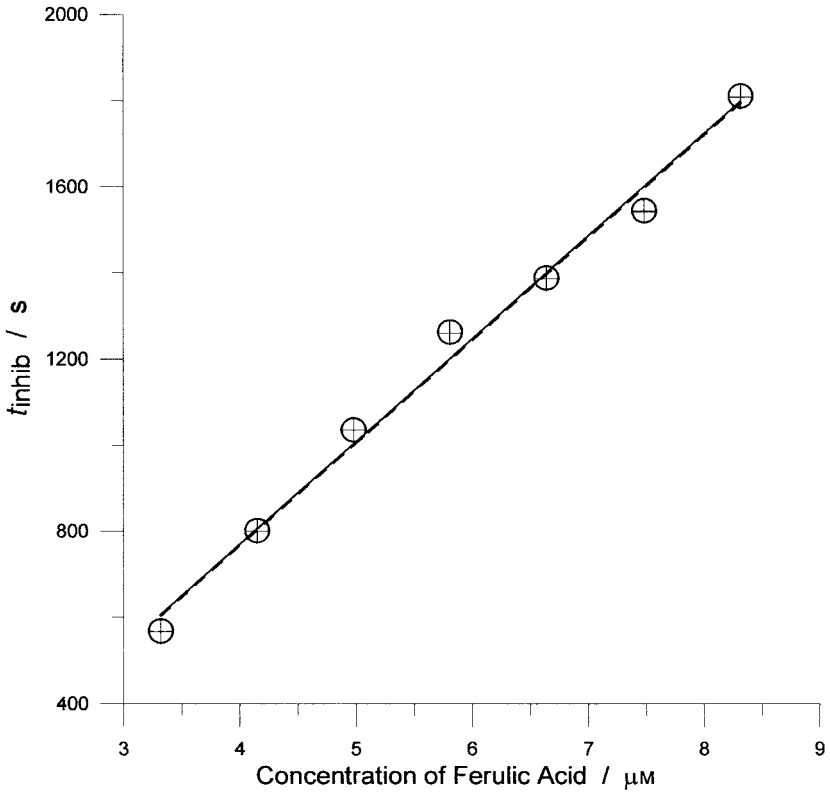

Fig. 6. *Behavior of the experimental* t_{inhib} *at* ca. 25° (○) *and of the corrected* t_{inhib} *at 25.0°* (+) vs. *the concentration of the antioxidant added* (ferulic acid)

inhibition times cannot be detected. We believe that, under these lower limits, the straight lines curve towards 0. At high concentrations of added antioxidant, the amplitude of the resumed oscillations becomes too low, until, up to a given concentration (different for each antioxidant), oscillations do not restart. This means that the reaction reached its end, being unable to produce radicals.

As can be seen from *Fig. 9*, the slopes of the straight lines are different (as in aqueous solution [2]), so the calculation of the relative antioxidant activity will depend on the concentration of the sample and on which substance is chosen as a standard. The parameters of the straight lines together with the *R*-squared values are reported in *Table 2*.

Cinnamic acid does not show inhibitory effects at concentrations up to 22 μM, this is probably due to the fact that it does not contain phenolic groups. It was not possible to try higher concentrations of cinnamic acid, because 22 μM is its solubility limit in the hydroalcoholic mixture.

Linearity between inhibition time *vs.* concentration for resorcinol (Re), hydroquinone, and *o*-cumaric acid was found in a narrow range of concentration, so these substances were not considered in the analysis.

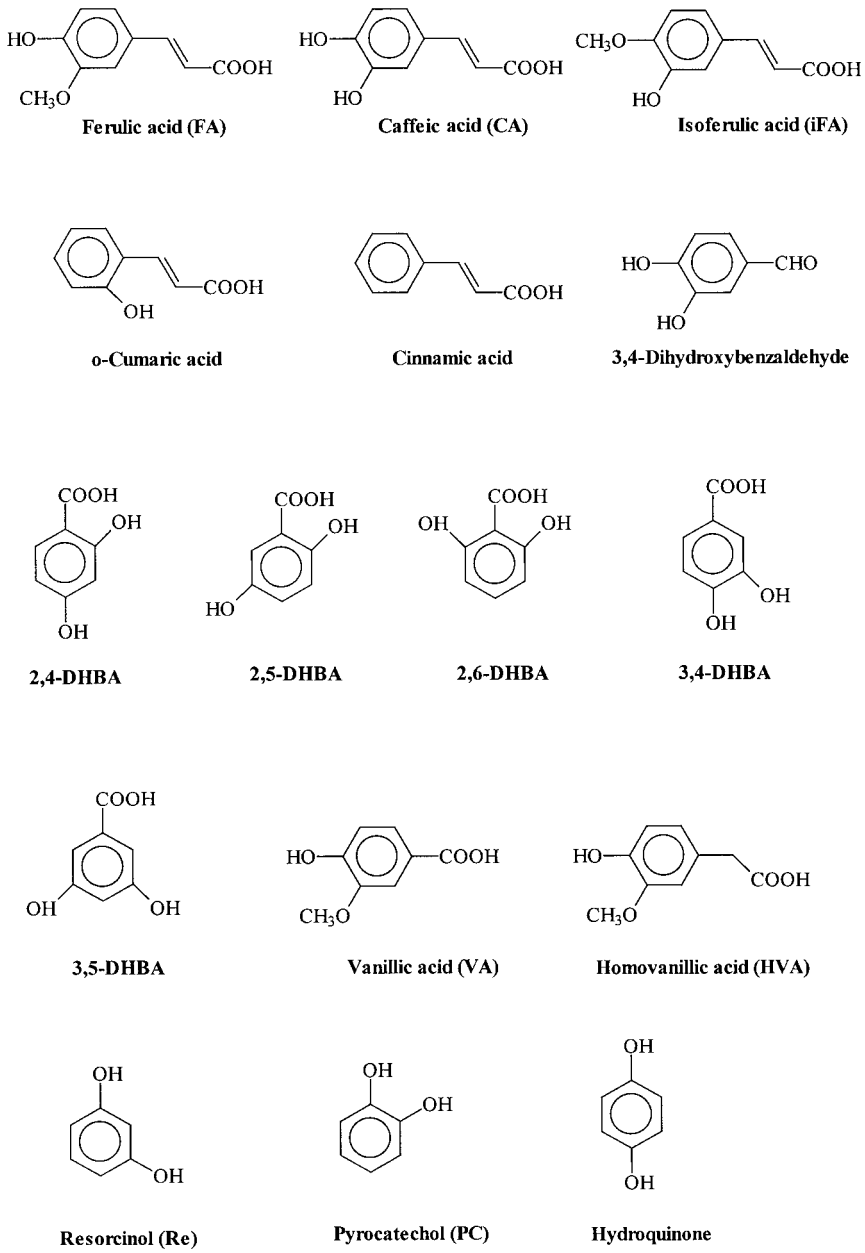

Fig. 7. *Structural formulae of the antioxidants studied*

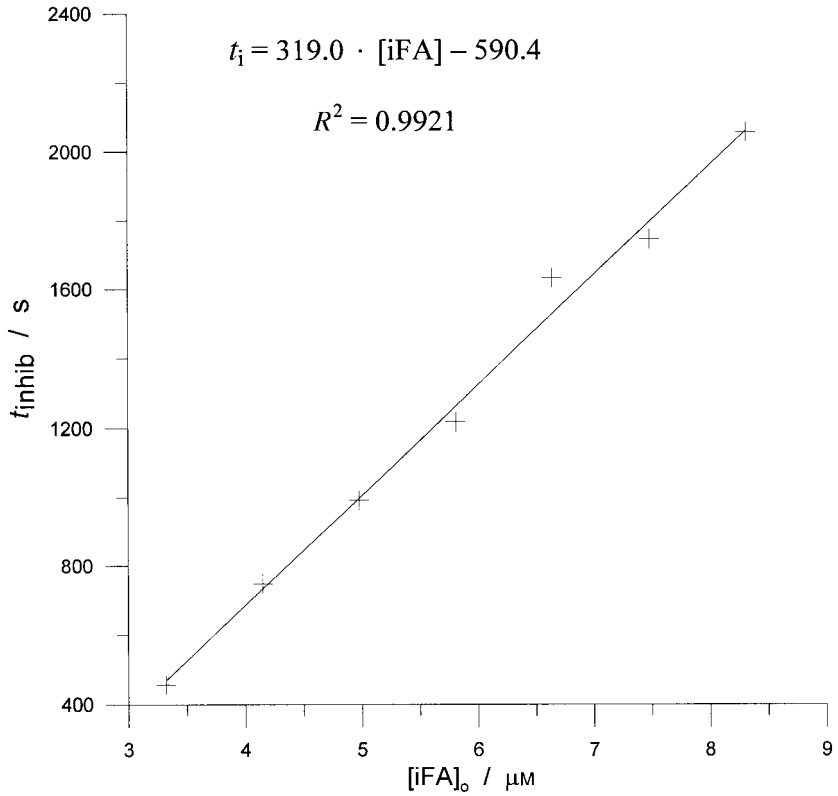

Fig. 8. *Straight line of* t_{inhib} *vs. concentration of isoferulic acid*

Relative antioxidant activities were calculated as r.a.c. (relative activity with respect to concentration) as defined in [2]:

$$\text{r.a.c.} = [\text{std}]/[\text{smp}]$$

where [smp] is the concentration of the sample in mixture, and [std] is the concentration of the standard that should give the same inhibition time. These concentrations were calculated from the straight-line equations of the substance chosen as sample and of the standard, respectively. The inhibition time must be specified together with the r.a.c. values. Caffeic acid (CA) was chosen as standard because the concentration intervals explored for almost all other antioxidants fall into the concentration interval explored for CA.

For six antioxidants, it has been possible to calculate a mean value of r.a.c. in the linear concentration range of the sample and the standard. This mean value, $(\text{r.a.c.})_m$, is more significant than the r.a.c. value calculated at only one inhibition time. The r.a.c. values obtained and $(\text{r.a.c.})_m$ are reported in *Table 3*. Quoted errors have been calculated by the procedure suggested by *Harris* [11].

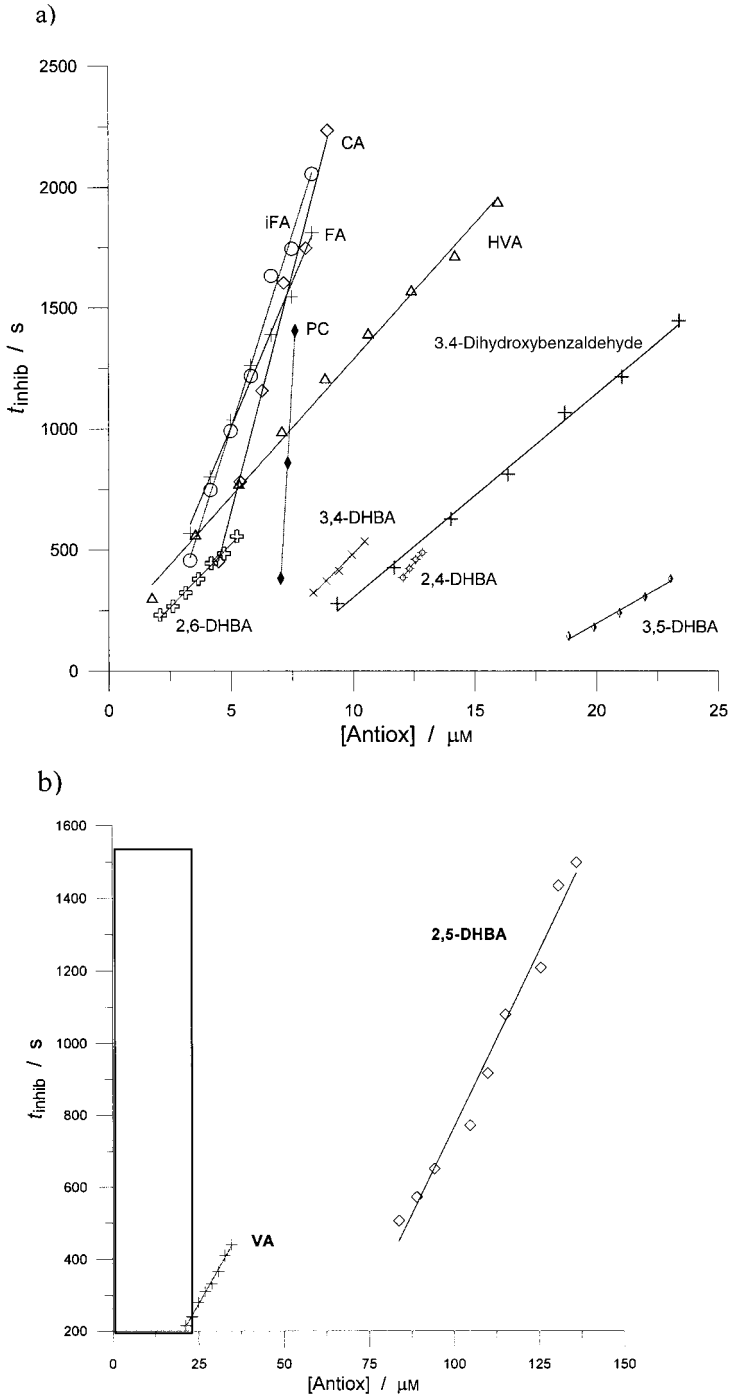

Fig. 9. a) *Straight lines of* t_{inhib} vs. *concentration in the range 2.5–25* μM *for several antioxidants studied.* b) *Straight lines of* t_{inhib} vs. *concentration in the range 25–150* μM *for 2,5-DHBA and VA*. The rectangle shows the plane portion in which the straight lines reported in *a*) fall.

Table 2. *Parameters of the Straight-Line Equations* ($t_{inhib} = m \cdot [\text{antioxidant}] + q$) *and R-Squared Values*

Antioxidant	m [μM^{-1} s]	95% Confidence limits on m	q [s]	95% Confidence limits on q	R^2
CA	391.2	$370.0 < \mu < 412.4$	−1298	$-1444 < \beta < -1152$	0.9894
FA	238.9	$229.6 < \mu < 248.2$	−186.9	$-243.1 < \beta < -130.7$	0.9912
iFA	319.0	$307.2 < \mu < 330.8$	−590.4	$-661.9 < \beta < -518.9$	0.9921
2,4-DHBA	131.2	$119.1 < \mu < 143.3$	−1192	$-1343 < \beta < -1041$	0.9933
2,5-DHBA	19.46	$18.64 < \mu < 20.28$	−1179	$-1270 < \beta < -1088$	0.9798
2,6-DHBA	104.3	$101.2 < \mu < 107.4$	+2.156	$-9.47 < \beta < 13.79$	0.9950
3,4-DHBA	101.3	$95.6 < \mu < 107.0$	−529.3	$-583.3 < \beta < -475.3$	0.9939
3,5-DHBA	57.36	$52.6 < \mu < 62.12$	−949.8	$-1049.7 < \beta < -849.9$	0.9868
3,4-Dihydroxybenzaldehyde	84.42	$82.38 < \mu < 86.46$	−540.9	$-575.5 < \beta < -506.3$	0.9966
HVA	113.0	$110.8 < \mu < 115.2$	+156.4	$134.7 < \beta < 178.1$	0.9956
PC	1734	$1608 < \mu < 1860$	−11819	$-12742 < \beta < -10896$	0.9992
VA	16.79	$16.41 < \mu < 17.17$	−142.8	$-153.6 < \beta < -132.0$	0.9955

As can be seen in some cases (2,5-DHBA, 3,4-dihydroxybenzaldehyde), r.a.c. values are the same (within the experimental errors) for different concentrations, but, in other cases, noticeable differences can be observed. Differences occur when slope and intercept of the standard line are different from those of the sample line.

For other antioxidants, it was possible to calculate only a single r.a.c. value at an inhibition time of 500 s. The results are reported in *Table 4*.

In *Table 4*, r.a.c. values at 500 s are reported also for some substances included in *Table 3*. The agreement between the $(\text{r.a.c.})_m$ and r.a.c. at 500 s values is satisfactory for 2,5-DHBA, 3,4-dihydroxybenzaldehyde, and iFA, while discrepancies can be noted for pyrocatechol (PC) and in particular for homovanillic acid (HVA). From a comparison of data reported in *Tables 3* and *4*, it can be seen that r.a.c. values at 500 and 600 s are the same within the experimental errors (with the exception of HVA).

Neglecting HVA because its anomalous behavior, the following ranking order of antioxidant capacity of the examined substances in mixed EtOH/H_2O medium can be obtained from data in *Tables 3* and *4:*

FA ≥ iFA > CA(std) > 2,6-DHBA > PC > 3,4-DHBA > 3,4-dihydroxybenzaldehyde ≈ 2,4-DHBA > 3,5-DHBA > VA ≫ 2,5-DHBA.

3. Discussion. – First, it was found that, at a parity of concentration, inhibition times in aqueous solution [2] are 2–3 times higher than those obtained in the hydroalcoholic medium for the substances studied here and in the previous work [2]. This may be due to a smaller amount of $HOO^{\bullet}$ radicals produced in EtOH/H_2O medium (probably caused by the smaller IO_3^- concentration) or to different rates for the reaction between antioxidants and radicals in the two media. In any case, it is reasonable to assume that an antioxidant (generally indicated as ArO–H) added to an active *BR* mixture subtracts $HOO^{\bullet}$ radicals *via* such a reaction as:

$$ArO{-}H + HOO^{\bullet} \rightarrow ArO^{\bullet} + H_2O_2 \text{ (ArO}^{\bullet}\text{ then decays to products)}$$

This was mechanistically proved for aqueous *BR* mixtures in the previous paper [2].

Table 3. *Relative Activities with Respect to Concentrations, r.a.c., and (r.a.c.)$_m$*

Antioxidant	t_{inhib} [s]	[Smp] [μM]	[Std] [μM]	r.a.c.	$(r.a.c.)_m$
FA	600	3.29	4.85	1.47 ± 0.05	
	800	4.13	5.36	1.30 ± 0.05	
	1000	4.97	5.87	1.18 ± 0.04	1.22 ± 0.09[a])
	1200	5.81	6.39	1.10 ± 0.04	
	1400	6.64	6.90	1.04 ± 0.04	
iFA	600	3.73	4.85	1.30 ± 0.05	
	800	4.36	5.36	1.23 ± 0.04	
	1000	4.99	5.87	1.18 ± 0.04	1.19 ± 0.04
	1200	5.61	6.39	1.14 ± 0.04	
	1400	6.24	6.90	1.11 ± 0.04	
2,5-DHBA	600	91.42	4.85	0.053 ± 0.002	
	800	101.70	5.36	0.53 ± 0.002	
	1000	111.97	5.87	0.052 ± 0.002	0.052 ± 0.003
	1200	122.25	6.39	0.052 ± 0.002	
	1400	132.53	6.90	0.052 ± 0.002	
3,4-Dihydroxybenzaldehyde	600	13.51	4.85	0.36 ± 0.01	
	800	15.88	5.36	0.34 ± 0.01	
	1000	18.25	5.87	0.32 ± 0.01	0.33 ± 0.01
	1200	20.62	6.39	0.31 ± 0.01	
	1400	22.99	6.90	0.30 ± 0.01	
HVA	600	3.93	4.85	1.23 ± 0.04	
	800	5.70	5.36	0.94 ± 0.03	
	1000	7.47	5.87	0.79 ± 0.03	0.86 ± 0.12
	1200	9.24	6.39	0.69 ± 0.02	
	1400	11.01	6.90	0.63 ± 0.02	
PC	600	7.16	4.85	0.68 ± 0.02	
	800	7.28	5.36	0.74 ± 0.03	
	1000	7.39	5.87	0.79 ± 0.03	0.79 ± 0.05
	1200	7.51	6.39	0.85 ± 0.03	
	1400	7.62	6.90	0.91 ± 0.03	
CA	600	–	4.85	1	
	800	–	5.36	1	
	1000	–	5.87	1	1
	1200	–	6.39	1	
	1400	–	6.90	1	

[a]) Standard error of the mean.

Table 4. *Relative Activities with Respect to Concentrations at $t_{inhib} = 500$ s*

Antioxidant	t_{inhib} [s]	[Smp] [μM]	[Std] [μM]	r.a.c.
iFA	500	3.42	4.60	1.35 ± 0.05
2,4-DHBA	500	12.90	4.60	0.36 ± 0.01
2,5-DHBA	500	86.28	4.60	0.053 ± 0.002
2,6-DHBA	500	4.77	4.60	0.96 ± 0.03
3,4-DHBA	500	10.16	4.60	0.45 ± 0.02
3,5-DHBA	500	25.28	4.60	0.18 ± 0.006
3,4-Dihydroxybenzaldehyde	500	12.33	4.60	0.37 ± 0.01
HVA	500	3.04	4.60	1.51 ± 0.05
PC	500	7.10	4.60	0.65 ± 0.02
VA	500	38.29	4.60	0.12 ± 0.004

It is possible that the antioxidants undergo also other reactions like oxidation or iodination, but we believe that the scavenging of the $HOO^{\bullet}$ radicals is the main reason for the inhibition of oscillations under the described experimental conditions.

To make a comparison between the ranking orders of antioxidant capacity in mixed $EtOH/H_2O$ medium and in aqueous solution for several substances studied, we suitably transformed data reported in the second column of Table 2 of [2] into values referred to CA as a standard. The r.a.c. order of activity obtained in aqueous solution referred to CA is:

$$FA \approx CA\ (std) > PC > 2,6\text{-DHBA} > 3,4\text{-DHBA} > 2,4\text{-DHBA} \approx 3,5\text{-DHBA} \gg 2,5\text{-DHBA}$$

Comparing this ranking order with that obtained for the same antioxidants in mixed $EtOH/H_2O$ medium reported in the previous section, a satisfactory agreement between the orders of activity in either aqueous or mixed medium[2]) can be seen.

Recently *Schlesier et al.* [12] published the assessment of the antioxidant activity by using six different *in vitro* methods. The six common tests for measuring antioxidant activity (TEAC I – III [13], TRAP [14], DPPH [15], DMPD [16], PCL [17], and FRAP [18][3]) were evaluated by comparing the results of four antioxidants and applying the tests to some beverages. The assays differed in the pH (3.3 – 10.5) of the testing system and in the nature and type of production of radicals. Some assays are suitable for hydrophilic antioxidants, others for hydrophilic and lipophilic substances when the solvent of the system is changed. The results showed that these six methods were not comparable because the ranking order of the antioxidant activity of the examined antioxidants and beverages differed from assay to assay. Also, different solvent systems for the same assay led, in some cases, to different antioxidant activities. However, *Schlesier et al.* [12] concluded that, despite these differences, the results of these *in vitro* assays give an idea of the protective efficacy of antioxidants components of secondary plant products.

Our findings showed that there are no great differences in the ranking order of the relative antioxidant activity according to the *BR*-reaction method by a relatively small change in the solvent. Moreover, as stated in the *Introduction*, the *BR*-reaction method works at a pH value similar to that of the fluids in the human stomach. Since fruits and vegetables containing polyphenols are usually consumed by mouth, it is conceivable that they effect their first antioxidant capacity against free radicals produced in the stomach [19] and, in this way, prevent medical problems like cancer of this organ [20]. Thus, the *BR*-reaction method can give useful *in vitro* information on the antioxidant activity at low pH values, which had been difficult prior to the advent of this new physico-chemical method.

As far as antioxidants more lipophilic than those studied here are concerned, it is our intention to study the behavior of the *BR* reaction and inhibitory effects in mixed

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[2]) In the work reported in [2] the water-insoluble caffeic and ferulic acids were transformed into their soluble sodium salts.

[3]) *Abbreviations:* TEAC = trolox equivalent antioxidant capacity, TRAP = total radical-trapping antioxidant parameter, DPPH = 2,2-diphenyl-1-picrylhydrazyl, DMPD = *N*,*N*-dimethyl-*p*-phenylenediamine, PCL = photochemiluminescence, FRAP = ferric reducing ability of plasma.

organic/aqueous media (20% (*v*/*v*)) with other organic solvents as i-PrOH, THF, and MeCN where it has been reported that the well-known *Belousov* [21]-*Zhabotinsky* [22] reaction can occur [23].

Finally, for the study of the antioxidative activity some factors of the methods have to be considered: the practicability, instrumental requirements, and the time, expertise and cost necessary for the analysis. Concerning these aspects, the *BR*-reaction method has many advantages: the analysis is inexpensive and rapid, and the reagents and apparatus are commonly used in all chemical laboratories.

Experimental Part

Materials and Methods. Malonic acid (*Merck*, reagent grade, >99%), manganese(II) sulfate monohydrate (*Merck*, reagent grade, >99%), and $NaIO_3$ (*Merck*, reagent grade, >99.5%) were used without further purification. $HClO_4$ (*Merck*, 70–72%), H_2O_2 (*Merck*, 35–36.5%), abs. EtOH (*Merck*, 99.8%), and other chemicals were of anal. grade. $HClO_4$ was analyzed by titration *vs.* a standard 0.1M NaOH solution (from *Merck*). H_2O_2 was standardized daily by manganometric analysis. Aq. stock solns. were prepared from doubly distilled, deionized H_2O. Mixed EtOH/H_2O stock solns. were prepared employing a medium containing 20% (*v*/*v*) of the organic solvent. Antioxidants used: 2,4-DHBA (=2,4-dihydroxybenzoic acid; *Fluka*; reagent grade, ≥98%), 2,5-DHBA (*Aldrich*; reagent grade, 98%), 2,6-DHBA (*Aldrich*; reagent grade, 98%), 3,4-DHBA (*Acros Organics*; reagent grade, 97%), 3,5-DHBA (*Merck*; reagent grade, >98%), caffeic acid (=3-(3,4-dihydroxyphenyl)prop-2-enoic acid; *Merck*; reagent grade, >98%), ferulic acid (=3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid; *Aldrich*; reagent grade, >99%), isoferulic acid (=3-(3-hydroxy-4-methoxyphenyl)prop-2-enoic acid; *Merck*; reagent grade, >98%), 3,4-dihydroxybenzaldehyde (*Aldrich*; reagent grade >97%), vanillic acid (=4-hydroxy-3-methoxybenzoic acid; *Fluka*; reagent grade, ≥97%), homovanillic acid (=4-hydroxy-3-methoxybenzeneacetic acid; *Aldrich*; reagent grade, >98%), hydroquinone (=benzene-1,4-diol; *Lancaster*, reagent grade, >99%), resorcinol (=benzene-1,3-diol; *Fluka*; reagent grade, ≥98%), pyrocatechol (=benzene-1,2-diol, *Fluka*; reagent grade, ≥98%), *o*-cumaric acid (=3-(2-hydroxyphenyl)-prop-2-enoic acid; *Merck*; reagent grade, >98%), cinnamic acid (=3-phenylprop-2-enoic acid; *Aldrich*; 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*) couple, or the potential of a iodide-ion-selective electrode (*Orion*, model *9453*) – reference electrode couple (see above)[4]). Potentiometric measurements in the mixed EtOH/H_2O medium were often 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 suitable data-acquisition program Multi Achat II (WTW) has been used. The multimeter was equipped with a temp. sensor with an accuracy of ± 0.1°.

Potentials from the iodide-ion-selective electrode were transformed into concentrations (log [I^-], I^- being a fundamental intermediate in the *BR* system [3]) by means of a suitable calibration curve. The potential of the bright-Pt electrode – reference electrode couple (*i.e.*, the electromotive force of the mixture) depends on all the redox couples present in solution (I_2/I^-, Mn^{III}/Mn^{II}, I^{III}/I^{I}, I^{V}/I^{IV}, *etc.*) so the Pt-electrode potentials cannot be transformed easily to concentrations of a single component.

BR Mixtures in either aq. or mixed medium were prepared by dispensing the appropriate amounts of stock solns. of reagents with pipets or burets and mixing in a 100-ml beaker to a total volume of 30 ml. The order of addition was: malonic acid, $MnSO_4$, $HClO_4$, $NaIO_3$, and H_2O_2. Oscillations start after the addition of H_2O_2.

All solns. and reaction mixtures were maintained at constant temp. by means of a thermostating system (accuracy ± 0.1°). Inhibitory effects by antioxidants were studied by adding 1.0 ml of diluted hydroalcoholic soln. of antioxidant to 30 ml of an active *BR* mixture.

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[4]) It was observed that the electrodes (Pt and ion-selective electrodes) are not affected by the presence of relatively small percentages (20% (*v*/*v*)) of org. solvents, then the results are reproducible at 25–32° [23].

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