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COMPARATIVE STUDY ON QUANTITATIVE CHARACTERIZATION OF SPIN TRAPPING BY NITRONES

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ABSTRACT. The aim of this paper is the quantitative, comparative study of chemical structure dependence of free radical trapping capacity in the case of some different, but structurally related nitrones. This dependence hasn't been described yet in the literarure. The free radical trapping was studied mostly by ESR and polarographic methods. The obtained simulated spectra were in good agreement with the experimentally obtained ones. The structure of the ESR spectra showed a high dependence on the nature of applied solvent. The quantitative, structural evaluation of the free radical production was performed by polarographically induced Fenton reactions' parameters.

Key-words: *free radicals, nitrones, ESR spectra, polarography*

INTRODUCTION

It is well known [1,2] that the ESR spectra represent the first derivative of the absorbance vs. the intensity of the magnetic field. The interpretation of ESR spectra presumes the correlation of the experimental data with the structural parameters of the researched paramagnetic unit. The calculation of the g giromagnetic factor is one of the best ways to obtain a good result. The number of the observed bands in the hyperfine spectrum is higher than the number of equivalent protons with one unit, and their relative intensity corresponds to the coefficients of binomial expansions.

If a free radical contains more than one magnetic nuclei, each of them contribute to the splitting of spectrum according to Pascal triangle.

Since in this paper are prezented some results regarding the spectra of nitrones [3-5] of which structures contain an unpaired electron in the neighborhood of a nitrogen atom, so the spectrum splat into three (or more) lines with equal (or different) intensity, it is reasonable to start with some nitroxides are, as model molecules.

The tetramethylpypimidyl-N-oxyde (TEMPO) free radical (figure 1.) is often used as standard molecule [6]. In this case the number of spectral lines is (the nuclear spin of nitrogen is I = 1):

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$$N = 2x1x1 + 1 = 3$$

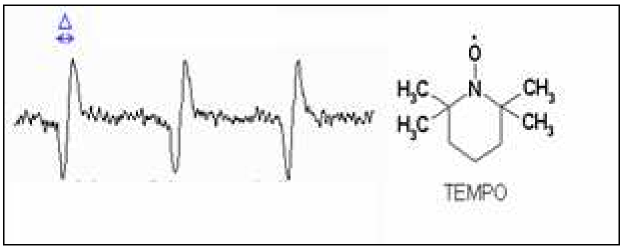


Figure 1. The ESR spectrum of tetrametylpyrimidyl-N-oxyde

The interaction with the 12 protons of the four methyl groups can be neglected. The stable diphenyl picrilhydrazyl (DPPH) free radical is also used as standard molecule [7]. In this case the two nitrogen atoms can be considered being equivalent, so the number of spectral lines (figure 2):

$$N = 2x2x1 + 1 = 5$$

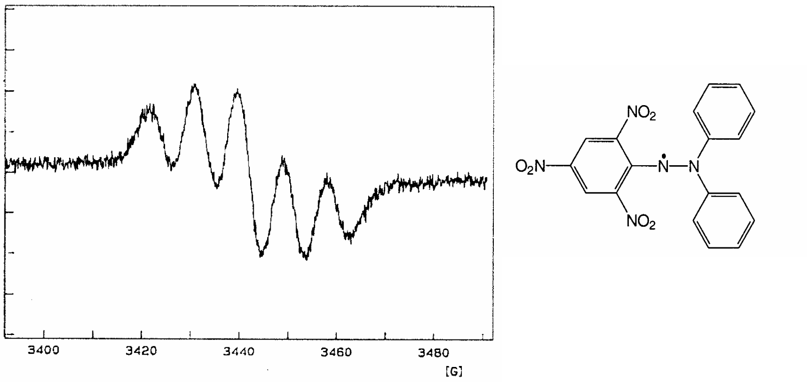


Figure 2. The ESR spectrum diphenyl picrilhydrazyl (DPPH) free radical

This specrum were verified experimentally by us, too.

RESULTS AND DISCUSSION

The spectrum of hydroxyl-diphenyl nitrone adduct can be explained in the same terms. The effect of the solvent must also be taken into account. The organic solvents usually give rise to broader, blurrier spectral lines. In ethanol/water or methanol/water mixtures besides the OH free radical in some of the cases appeared the hydroxyethyl and hydroxymethyl free radicals, as well. In case of diphenyl nitrones due to their bigger size comparing to the t-butylphenyl-nitrone, the rotation is slower, the spectral lines are broadened, so the resolution is poorer. As the nitrones are only slightly soluble in water, but easily soluble in organic solvents (methanol, ethanol, chloroform, dimethylsulfoxide), the ESR spectra were taken in different pure solvents, or mixture of them. Some example: the adduct of diphenyl nitrone in methanol (figure 3):

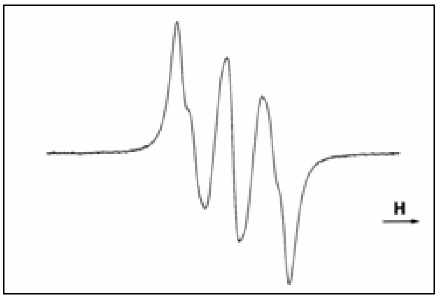


Figure 3. The ESR spectrum of the diphenyl nitrone adduct in methanol

in ethanol (figure 4):

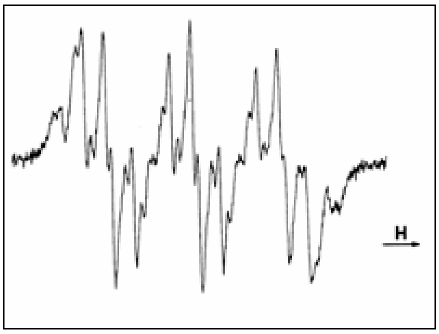


Figure 4. The ESR spectrum of the diphenyl nitrone adduct in ethanol

in dimethylsulfoxide (figure 5):

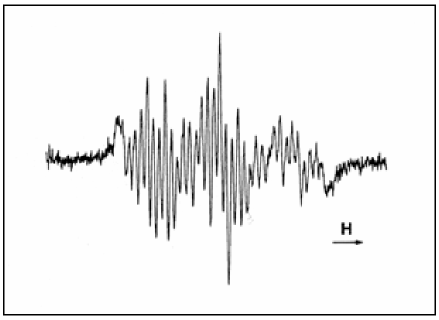


Figure 5. The ESR spectrum of the diphenyl nitrone adduct in dimethylsulfoxide

in ethanol/water mixture (figure 6):

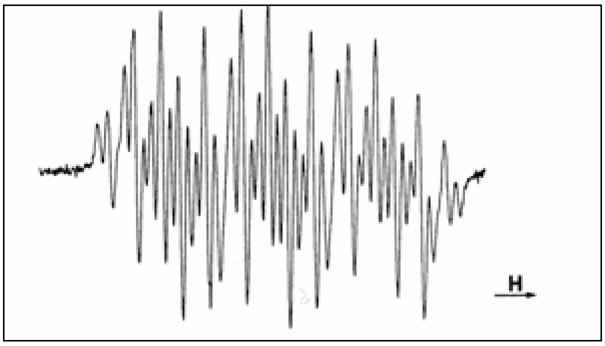


Figure 6. The ESR spectrum of the diphenyl nitrone adduct in methanol-water mixture

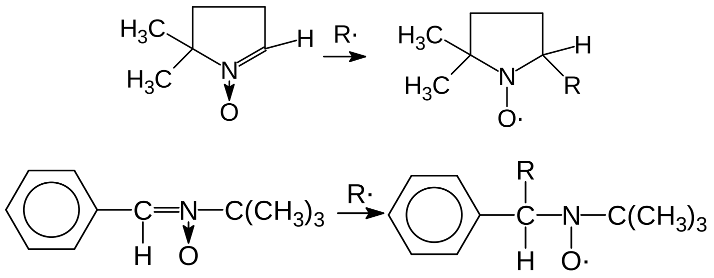
It can be seen that the spectrum taken in methanol is the most simple, but its disadvantage is that it does not reflect the hyperfine structure. Regarding this observation, the formation of other adducts must be taken into account besides the expected one – depending on the structure of the solvent – which makes the spectrum being much complicated [8]. Table 1. shows some examples of splitting constant is case of several free radicals captured by phenyl-butyl-nitrone.

Table 1. Splitting constants of several free radicals captured by phenyl-butyl-nitrone

The captured R• free radical	**The source of free radical**	**The splitting constants**	
		a_N (G)	a_H (G)
OH	H_2O_2 + UV	15.62	2.71
$\bullet CH_2OH$	$CH_3OH + H_2O_2$ + UV	16.06	3.87
$\bullet CH(CH_3)OH$	$C_2H_5OH + H_2O_2$ + UV	16.16	3.37
$\bullet C(CH_3)_2OH$	$(CH_3)_2CHOH + H_2O_2$ + UV	16.12	3.61
$\bullet CH_3$	DMSO + H_2O_2 + UV	16.47	3.57
$\bullet OCH_3$	DMSO + H_2O_2 + air + UV	15.13	3.37

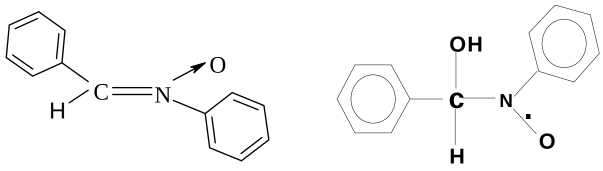
The ESR spectra of some other, NO-centered free radicals derived from nitrones are relatively similar to the above presented ones, but – naturally – dependent on the structure of R• free radical (Scheme 1).

The same free radicals are formed in the Fenton reactions' condition.



Scheme 1. Structures of the studied nitrones

Diphenyl nitrone captures mostly the HO• free radicals (Scheme 2):

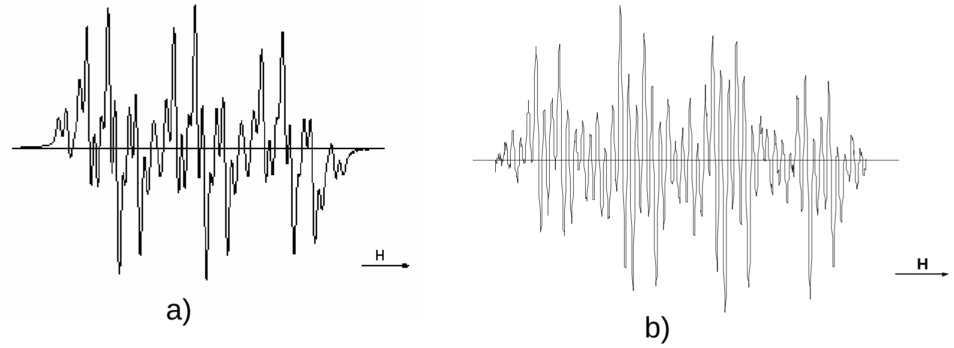


Scheme 2. The capture of free radicals by diphenyl nitrone.

The g-factor of diphenyl nitrone's hydroxyl adduct can be calculated using the g factor value of dipicrylhydrazyl etalon, by the following proportionality:

$$g = g_{etalon}B_{etalon}/B_{sample}$$

The obtained value is specific to the nitoxyde-type free radical. Compared to the theoretically obtained parameter, the match is not perfect, which can be explained by unique feature of concrete compunds. Figure 7. shows the ESR spectra of some diphenyl nitrone derivatives synthesized by us.



a)

b)

Figure 7. The ESR spectrum of the studied nitrones in methanol-water a) and ethanol-water b) mixture

The hydroxyl spin adduct's life time is about 16 hours, the concentration of the free radicals can be traced approximatively after 3 hours.

Our aim was the to describe the comparative and quantitative study of free radical trapping capacity of different, but structurally related nitrones [4]. Due to the organic solvents the spectral lines are broadened, so only the coupling provenient of N-atom and β-hydrogen was detectable. The fundamental spectrum in methanol is triplet, and in ethanol is quartet.

The spectra could be reproduced with good agreement by simulation. During the interpretation of these spectra several questions arose. One of the questions is, how can be interpreted the interaction of three equivalent protons with the unpaired electron, which influences the primary coupling (3.45 G) of the α-proton with a splitting of a_H = 2.8 G. The interaction suppose a dynamic structure, the static structure does not show which are the protons that – because of the quick molecular movement – are „seen" being magnetically the same by the 2p electrons localized on the N atom. This rotation could cause the unexpected interaction with three protons.

It was revealed, that the spectra of C-3,4-dimetoxy-derivative and the unsubstituted diphenyl nitrone are practically the same, but the those of the C-2,3,4-trymetoxy-nitrone is somewhat different. In the first two cases the orto position is free. Regarding the electronic effects, the metoxy-group causes shifts: from meta- position it has an electron attractive, from para- position it has a repulsive effect. So the simultaneous presence of the two groups diminishes the overall effect. In case of spin trapping, the free radical attacks the azomethinic carbon, the C=N double bond is cleaved, and the conjugation is ceased. In case of single bonds the electronic effects decay in approximately two bond-lengths, so the effect does not reach to the nitrogen. The effect of a substituent in orto- position is about the same intensity as of the para- position, but because of the steric impediment the ring is contortioned, and the conjugation is ceased before the attack of free radical, moreover, the coupling of free radical to the carbon atom is also more difficult. Finally, the conjugation is ceased in both of the cases, so the nitrogen atom is affected always by the same coupled group (captured free radical) [5].

The ESR spectra can be interpreted clearly only in the simplest cases. The physico-chemical information is often masked by different factors, especially when more paramagnetic nuclei are present. That is the reason why is important to generate the simulated spectra, to compare the spectral parameters. For this purpose the EasySpin (Matlab) software package is appropriate. The experimental spectrum taken in metanol-water mixture was compared with the simulated one (figure 8). The match is striking. The effect of the meta-positon protons of the N-coupled aromatic ring is negligible. All of this describe the structure of the adduct in the way above indicated.

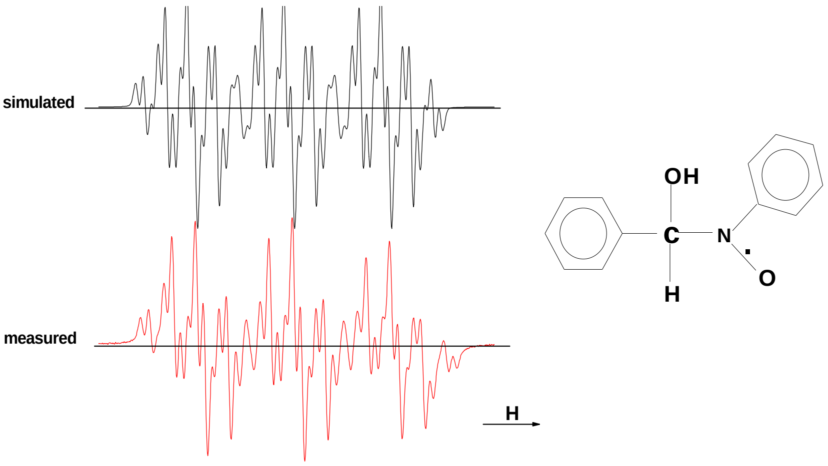


Figure 8. Comparison of the experimental and simulated spectra

In ethanolic medium also the hydroxyethyl adduct is formed (figure 9).

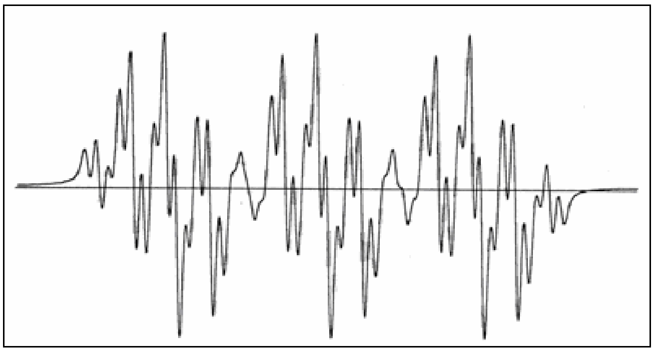


Figure 9. The ESR spectrum of hydroxyethyl spin adduct by diphenyl nitrone spin trap

In figure 10 the ESR spectrum of 2,3,4-trimetoxy-C-phenyl-nitrone can be seen. Because of substitution of orto-H, the spectrum is somewhat different from the unsubstituted basic nitrone.

In the following session for describing the free radical source and trapping will use the electrochemical analogy. As in the redox processes the electrons always passes from one reactant to other one by one, this process can be considered being analogue with the attack of free radicals. In the complex mechanism of the electrode processes one or more chemical steps could be inserted, so most of these transformations are of chemical/ electrochemical nature (CE, EC, ECE, etc.). The analogy between the chemical and electrochemical reactions can be generalized as follows (scheme 3.) [9,10]:

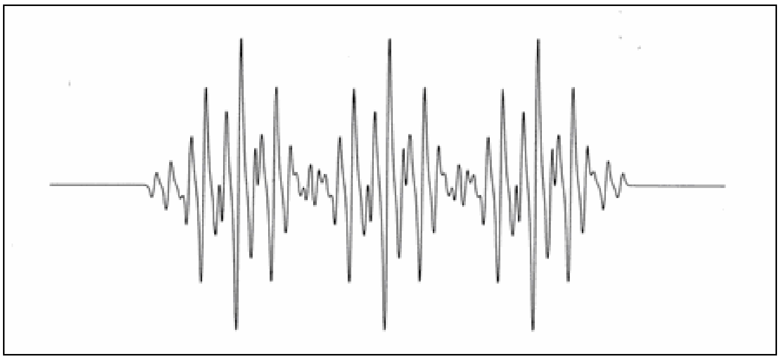
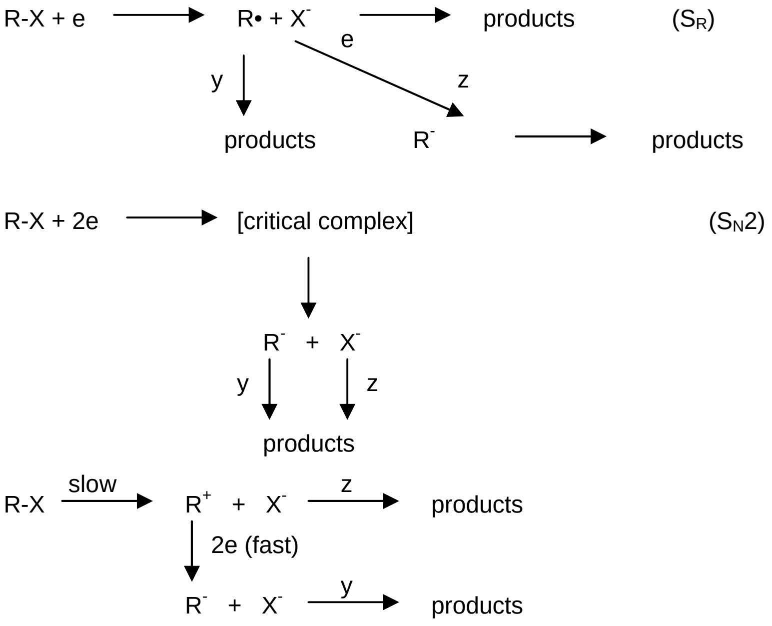
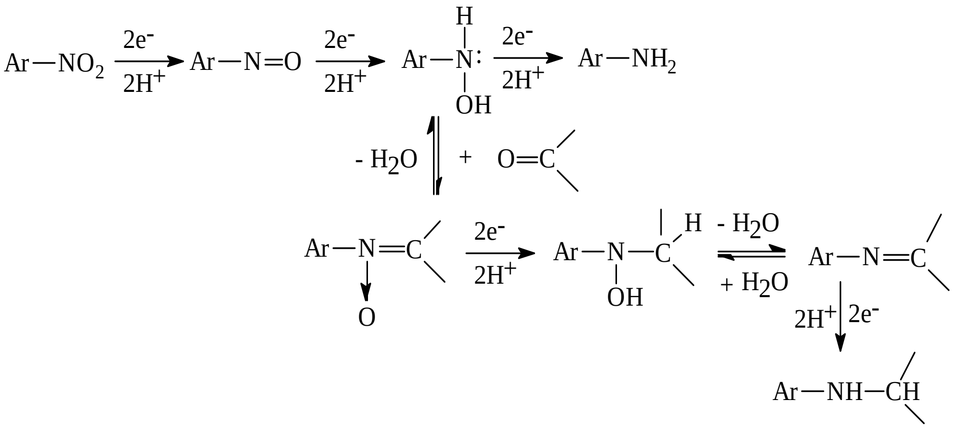


Figure 10. The ESR spectrum of 2,3,4-trimetoxy-C-phenyl-nitrone



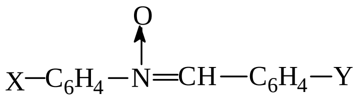
Scheme 3. The analogy of chemical and electrochemical reactions



Scheme 4. The synthesis of the studied nitrones

This system also contains the free radicalic transformation. Based on the analogy of unpaired electron-containing free radical and cathodic attack, the electrochemical (polarographic, cathodic) reduction of several hundreds of substituted diphenyl nitrones was done by us. The half-wave potentials differ significantly in case of meta- and para-substituted derivatives. In this experiment the whole nitrone-molecule constituted a single conjugate system, therefore was nearly coplanar (scheme 4.) [11-13].

The general formula of the two ring-substituted diphenyl nitrone depolarizers was (scheme 5):



$$X{-}C_6H_4{-}\overset{\overset{O}{\uparrow}}{N}{=}CH{-}C_6H_4{-}Y$$

Scheme 5. The general formula of the two ring-substituted diphenyl nitrone

The X and Y orto-, meta- or para-substituted diphenyl nitrones allowed the tracing of the structure effect during the polarographic reduction of oxyazomethine group. The effect of substituents was taken into account via σ constants. The trap in the free radical capture determinations was also the nitrone group. These measurements were done on C-phenyl-substituted derivatives. Firstly the X = H and Y was H (unsubstituted basic compound), then NH_2, OH, Cl, $N(CH_3)_3$, $NHCOCH_3$, in orto-, meta- and para- positions. Their effect could be described with the following linear equation:

$$E_{1/2} = (0.098 \pm 0.031)\,\sigma - (0.925 \pm 0.007)$$
$$N = 10,\ r = 0.990$$

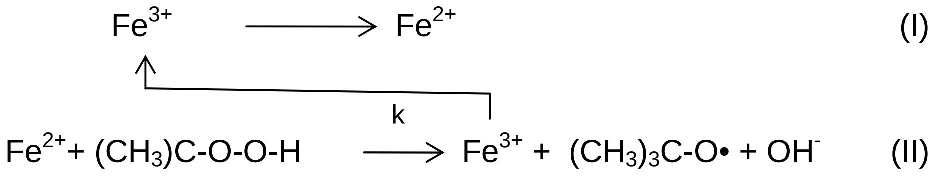
The half-wave potentials characterize the electron transfer (electron capture) energy. The ESR data and the polarographic parameters are completing each other reciprocally.

Our team proposed a method based on the kinetic polarographic currents for quantitative characterization of free radical activity and the trapping capacity. The method has the following idea. The literature propose principally the Fenton reaction (Fe(II) + H_2O_2) for obtaining free radicals, but better is the tert.-butyl-hydroperoxide (TBH), which has already been used for characterization and comparison of many free radicals. In this case the question is, what is the trapping capacity of a given compound (ex. the basic diphenyl nitrone) against free radicals with different structure. And, besides the structure, the solvent has a major effect. After the first works of Wiesner the possibility of polarographic tracing of very fast reactions ($k \approx 10^3 - 10^{10}\ s^{-1}$) was contoured. This goal can be reached by the kinetic polarographic currents [14]. The corresponding theoretic problems were solved by the Prague school.

In complex electrode processes the mass transport is ensured by diffusion. But, under certain conditions, this transport is influenced by chemical reactions which, together with the diffusion, contribute to balance the concentration-changes due to the electrolysis in the neighborhood of the electrode. In consequence, the polarographic currents may depend on the speed of the mentioned reactions. These currents are called kinetic currents.

The reactions between organic hydroperoxides and transition metals (especially Fe^{2+}) were thoroughly studied lately. These redox systems (Fenton reactions) are free radical sources widely used both in basic research and applicative one. From this point of view, the speed of reaction between peroxide and metallic ion is decisive. In present, only the reactions of cumene-hydroperoxide and the TBH with transition metals are studied.

Our team studied the kinetics and mechanism of the reaction between TBH (the typical representative of hydroperoxides) and Fe^{2+} ions. Considering TBH the benchmark of the free radical sources, it becomes possible the numerical determination of the free radical trapping capacity of different compounds (ex. nitrones). In the polarographic cell, under certain conditions, when the Fe^{3+} ions are reduced at less negative potential than the hydroperoxide ($E_{1/2,Fe3+}$ = – 0.265 V, against $E_{1/2,ROOH}$ = - 0.660 V, vs. NSE, in 1.0 M H_2SO_4), the Fe^{2+} ions formed are partially regenerated by the hydroperoxide, so the limiting current of the Fe^{3+} ions increases. The increase in current depends on the rate of reaction between hydroperoxide and Fe^{2+} ions, which means that its value allow the calculation of the rate of free radical trapping reaction. The principal steps of the depolarisation process can be described by the following scheme (scheme 5):



Scheme 5. The principal steps of the depolarisation process

The $(CH_3)_3C\text{-}O\bullet$ can be coupled to a free radical trap, or can initiate a chain reaction depending on the composition of the system.

Koutecký demonstrated that the ratio of the average total current density and the average diffusion current density can be described by the following asymptotic equation:

$$\frac{\bar{i}_l}{\bar{i}_d} = 0.812(kCt_1)^{\frac{1}{2}} + 1.92(kCt_1)^{-\frac{7}{6}}$$

where C is the concentration of hydroperoxide.

For a correct interpretation of the results the ratio of the oxidized Fe^{2+} and consumed $(CH_3)_3C\text{-}OOH$ moles (R) must be known. The experimentally obtained value in our case was R ≈ 1.0, which indicates that one hydroperoxide molecule oxidize only one Fe^{2+} ion, according to the proposed reaction scheme.

It is resulting that the reaction between Fe^{2+} and TBH is of first order referring to both partners. This stoechiometric relation differs in case of reaction between Fe^{2+} and HOOH, which was found to be R = 2. Our experimental results are presented in the table 2. ($[Fe^{2+}] = 1.0\times10^{-3}$ M]:

Table 2. The experimental results

[HTB] (M)	$\bar{i}_l / \bar{i}_d$	t_1(s)	k ($M^{-1}s^{-1}$)	$\bar{i}_l / \bar{i}_d$	t_1(s)	k ($M^{-1}s^{-1}$)
	T = 303 K,			T = 298 K,		
0.045	1.818	2.86	30.13	1.657	3.17	20.93
0.040	1.700	2.70	29.83	1.539	3.04	19.06
0.030	1.645	3.10	31.45	1.441	3.15	19.70
0.020	1.391	2.70	30.07	1.244	2.72	17.91
$\bar{k} \pm S_M$	30.37 ± 0.36			19.53 ± 0.62		
	T = 293 K,			T = 288 K,		
0.045	1.460	3.22	13.47	1.281	3.26	7.72
0.040	1.443	3.24	14.50	1.263	3.30	8.05
0.030	1.303	3.20	12.78	1.196	3.25	7.88
0.020	1.183	2.80	12.79	1.112	2.83	7.63
$\bar{k} \pm S_M$	13.38 ± 0.40			7.77 ± 0.10		

$$\lg k = (3395 \pm 140)1/T + (12.68 \pm 0.45)$$
$$r = -0.998 \qquad s_0 = \pm 0.018$$

or

$$k = 4.8\text{x}10^{12} \exp\left(-\frac{15500}{RT}\right) \ M^{-1}s^{-1}$$

The activation parameters are: $\Delta H^{\neq}_{298}$ = 14.9 ± 0.6 kcal/mol; $\Delta S^{\neq}_{298}$ = -2.5 ± 2.1 cal/molK; $\Delta G^{\neq}_{298}$ = 15.6 ± 0.6 kcal/mol.

This rate is 3-4 times smaller as those of the reaction between Fe^{2+} and H_2O_2. The kinetic data regarding the reaction of TBH with Fe^{2+} are missing from the literature. It is foreseen that the value of catalytic current decreases when a free radical trap is added to the system. For this purpose we used nitrones in our experiments. The decrease of catalytic wave height can be explained by interaction between the HO• free radicals and the trap. In case of a sufficiently high concentration of spin trap the catalytic current density approaches to a limit value:

$$\frac{\bar{i}_l}{\bar{i}_d} = 0.812(k[H_2O_2]t_1)^{\frac{1}{2}}$$

which, compared to the value observed is absence of the spin trap:

$$\frac{\bar{i}_l}{\bar{i}_d} = 0.812(2k[H_2O_2]t_1)^{\frac{1}{2}}$$

is about 30% less, according to the:

$$Fe^{2+} + HOOH \rightarrow Fe^{3+} + HO^{-} + {\bullet}OH$$

reaction.

CONCLUSIONS

Taking into account the presented results it becomes obvious that the ESR spectra can provide basic information regarding the spin trapping capacity of nitrones, the kinetics and mechanism of spin trapping.

Using polarographic kinetic data the free radical producing capacity of some free radical sources can be obtained.

The results obtained from magnetic spectra and those obtained from the electrochemical methods are mutually complementary in study of free radical interactions.

EXPERIMENTAL SECTION

The ESR and the polarographic studies were made by a method described earlier by us [9-11], using a Polarograph LP-55, system Heyrovsky, connected to a recorder EZ-2 and an ultrathermostat type Höppler. Respectively, the components were mixed and filled into a flat cell (Wilmad, USA, type WG-808 Q), and the ESR spectrum recorded.

The data used for simulations were: number of components: 1; resolution of the magnetic field: 0.05; number of coupling constants: 4; particular line width: 0.60 G; the Lorenz/Gauss factor: 0.75/0.25; microwave frequency: 9.756 GHz; magnetic induction: H_0: 3482.0 G; Sweep: 50.0 G; g-factor: 2.0027; the hfs. coupling of N atom: a_N = 12.26 G; the a hfs. coupling of Hα proton: a_H = 3.45 G; the a hfs. coupling of the three a equivalent H(3) protons: a_H (3) = 2.80 G; the hfs. coupling of the two equivalent H(2) protons: a_H (2) = 0.90 G.

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