

DEPENDENCE OF THE ESR SPECTRA INTENSITY OF $POBN : O_2^{\bullet-}$ ADDUCT ON THE CONCENTRATIONS COMPONENTS IN NON-FENTON REACTION

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Abstract. In the paper the trapping of the $O_2^{\bullet-}$ radical by the nitron α -(4-pyridyl-1-oxide)-N-tert-butyl nitron (POBN) compound was investigated. The $O_2^{\bullet-}$ radicals were generated by the non-Fenton reaction of water solutions of KOH and H_2O_2 reagents. The ESR spectrum of $POBN : O_2^{\bullet-}$ adduct can be recorded under carefully selected concentration of components. The best spectra were obtained for 6% KOH and 6% H_2O_2 .

Keywords: Non-Fenton generation of $O_2^{\bullet-}$, ESR spectra of $POBN : O_2^{\bullet-}$.

Zależność intensywności widma ESR adduktu $POBN : O_2^{\bullet-}$ od stężeń składników reakcji niefentonowskiej

Streszczenie. W pracy badano pułapkowanie rodnika $O_2^{\bullet-}$ przez α -(4-pyridyl-1-oxide)-N-tert-butyl nitron (POBN). Rodnik $O_2^{\bullet-}$ generowano w reakcji niefentonowskiej z KOH i H_2O_2 . Widmo ESR adduktu $POBN : O_2^{\bullet-}$ powstaje po starannym dobraniu stężeń substratów. Najlepsze widmo otrzymuje się dla stężeń 6% KOH i 6% H_2O_2 .

Słowa kluczowe: Nie Fentonowskie generowanie rodnika $O_2^{\bullet-}$, widmo ESR adduktu $POBN : O_2^{\bullet-}$.

Introduction

Free radicals are chemical paramagnetic species having a non-paired electron. The most useful method of its detection is the electron spin resonance (ESR) [1]. In medicine and biology the hydroxyl HO^{\bullet} and peroxide anion radical $O_2^{\bullet-}$ are particularly important and are widely studied because they play a crucial role in the life and defense processes of cells as well as they are responsible for pathology cases in medicine [2, 3]. In biological cells the most important sources of radicals is the transport of electrons for example in photosynthesis. The leakage of electrons from this chain causes the reduction of oxygen species.

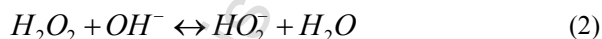
In vitro the hydroxyl radicals are created in the Fenton reaction [4, 5]. On the contrary, the peroxide radicals are products of the non-Fenton reaction [6, 7].

The living time of the wide group of free radicals is a short period ranged from a few nanoseconds up to a few minutes. The peroxide anion radical is a very short-lived oxidant specie and therefore, it can be investigated by ESR using the spin trapping method [7]. In this paper we present such measurements using POBN trap for different concentrations of components in the H_2O_2 / KOH / DMSO system.

The aim of the studies was experimental determination such relative concentration of the components at which the intensity of ESR signal is the best.

1. Superoxide anion-radical formation in the non-Fenton reaction

During the first stage in the chain of triplet oxygen reduction the superoxide anion-radical $O_2^{\bullet-}$ is formed is a product [8]. The non-Fenton generation of superoxide anion-radical is described with the following equations:



2. Spin trapping of $O_2^{\bullet-}$ and the spectrum of $POBN : O_2^{\bullet-}$ adduct

In Fig.1a) the scheme of the α -(4-pyridyl-1-oxide)-N-tert-butyl nitron (POBN), compound is presented. In this trap the free electron is localized at the N-O group. The single chemical binding of the nitrogen atom with carbon atom gives the opportunity for binding of the trapped radical to the carbon atom with simultaneously breaking the double C=N binding to the C – N binding. The presence of the substitutes gives rise to the stabilization of the trap in the radical state. The general scheme of trapping

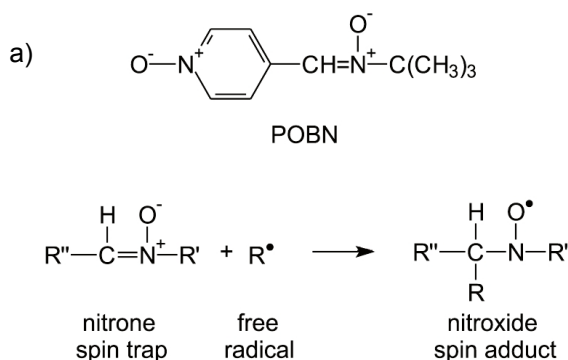


Fig. 1 a) Spin trap of nitron α -(4-pyridyl-1-oxide)-N-tert-butyl nitron (POBN),
b) General scheme of spin trapping [9]
Rys. 1 a) Pułapka spinowa α -(4-pyridyl-1-oxide)-N-tert-butyl nitron (POBN),
b) ogólna reakcja pułkowania spinowego [9]

The ESR spectrum of the $POBN : O_2^{\bullet-}$ adduct reflects hyperfine structure of the energy levels that arises from the interaction of electron spin $s = \frac{1}{2}$ with two hydrogen and nitrogen nucleus spins $I_H = \frac{1}{2}$ and $I_N = \frac{3}{2}$. The constants a_N and a_H reflects the magnitude of the superfine splitting. As a result, the doublet triplet is formed. The hyperfine energy levels splitting of ESR spectrum is presented in Fig. 2.

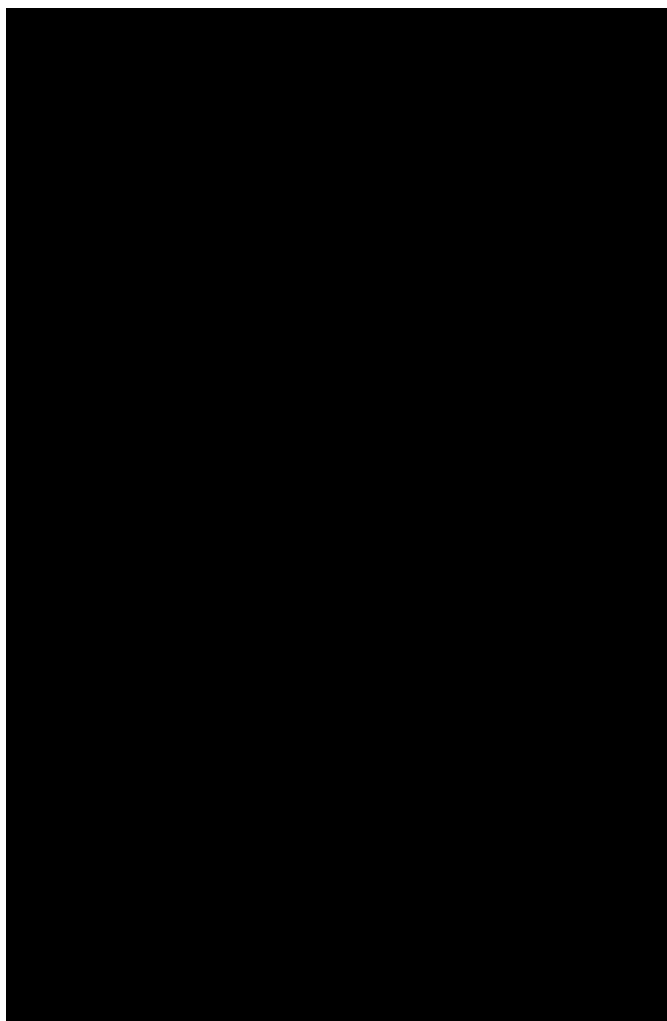


Fig. 2. a) Hyperfine $H_{SI_H} + H_{SI_N}$ energy splitting, b) the ESR spectrum of $POBN : O_2^{*-}$ adduct

Rys. 2. a) Struktura nadsubtelna rozszczepienia energii $H_{SI_H} + H_{SI_N}$.
b) widmo ESR adduktu $POBN : O_2^{*-}$

3. Experimental details

The solutions for generation of superoxide anion-radicals were prepared from calcium hydroxide and hydrogen peroxide, both of the spectroscopic grade. All solutions were made using redistilled water. Spin trapping agent was POBN purchased from Aldrich (99%) and it was dissolved in dimethylsulfoxide DMSO obtained from Polish Company POCH (99 %). All reagents were dissolved in the glass cell and were stored in bromine-silicate bottles preserving good chemical and thermal proof.

In the Table 1 the solutions concentrations are listed. In the aim to obtain 0.18 M/dm^3 POBN solution $1 \square 10^{-4} \text{ l}$ of 99% POBN and $1 \square 10^{-4} \text{ l}$ of DMSO were mixed. The material of the system $H_2O_2 / KOH / DMSO/POBN$ were placed in the quartz micro test-tube for ESR measurements ($\phi = 4 \square 10^{-3} \text{ m}$, $l = 1 \square 10^{-2} \text{ m}$). The temperature of studies was room temperature (293K). The SE/X-10 ESR spectrometer with double modulation of the field, working in X band (9.5GHz) was used. The spectrometer was connected with a computer via LabVIEW system [10].

Table 1. The combinations of solutions concentration
Tabela 1. Kombinacje stężeniowe roztworów

POBN + DMSO solution A	H_2O_2 solution B	KOH solution C
0,18 M/dm ³	4%	6%
	6%	4%
	6%	6%
	6%	10%
	6%	15%
	9%	6%
	12%	6%

4. Results

In Fig 3 and 4 the dependences of ESR spectra of $POBN : O_2^{*-}$ - adduct on the concentration of non-Fenton reagents are presented.

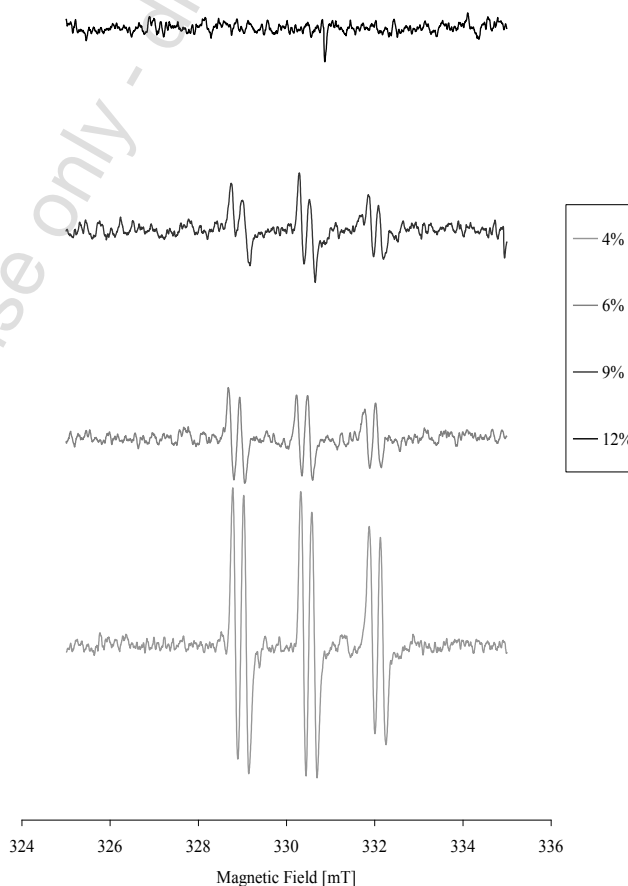


Fig. 3. Effect of concentration of the KOH solution on the EPR spectrum, with a steady 6% concentration of H_2O_2

Rys. 3. Wpływ stężenia roztworu KOH na widmo EPR, przy stałym 6% stężeniu H_2O_2

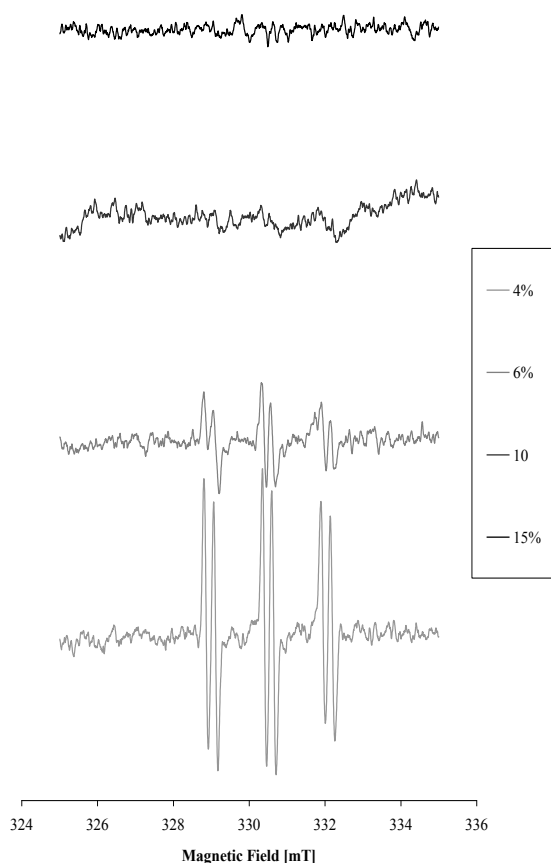


Fig. 4. Effect of concentration of the H_2O_2 solution on the EPR spectrum, with a steady 6% concentration of KOH

Rys. 4. Wpływ stężenia roztworu H_2O_2 na widmo EPR, przy stałym 6% stężeniu KOH

The spectra collected in Fig. 3 and 4, show that intensity of the ESR signal strongly depends on the concentration of the reagents.

We have determined constants a_N and a_H for various concentrations. The results are collected in Table 2.

Table 2. Calculated constants
Tabela 2. Obliczone stałe

Concentrations		a_H	a_N
H_2O_2	KOH		
6%	4%	0,252	1,544
6%	6%	0,245	1,549
6%	10%	-	-
6%	15%	-	-
4%	6%	0,246	1,542
9%	6%	0,292	1,547
12%	6%	-	-

5. Conclusions

It is known that the intensity of the EPR signal depends on the time between preparing the H_2O_2 / KOH / DMSO / POBN system and ESR recording. The g -factor, as well as a_N and a_H constants don't depend on the concentration of the H_2O_2 / KOH / DMSO / POBN system. The strongest signal is recorded for solutions with concentration 6% KOH and 6% H_2O_2 .

In this study we have placed the probe in microwave cavity at once. Time dependence of the spectra will be the subject of the next investigation.

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W pracy naukowej specjalizuje się w spektroskopii elektronowego rezonansu paramagnetycznego (EPR), w szczególności bada domieszki różnych jonów w kryształach laserowych i półprzewodnikach oraz wolne rodniki w lipidach i materiałach biologicznych. Jest autorem kilkudziesięciu artykułów z listy filadelfijskiej i materiałów konferencyjnych. Bierze czynny udział w grantach KBN, projektach PO IG oraz POKL. Jest członkiem organizacji: PTF, EPR/ESR Society, PG EMR gdzie pełni funkcję wiceprezesa.



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W pracy naukowej specjalizuje się w chemicznej analizie żywności przy zastosowaniu chromatografii cieczowej, chromatografii gazowej, spektrometrii mas, chromatografii sprzężonej ze spektrometrią mas, chemiluminescencji oraz spektrometrii paramagnetycznego rezonansu elektronowego.

