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Reaction of 2,2-Diphenyl-1-picrylhydrazyl with $HO^{\bullet}$, $O_2^{\bullet-}$, HO^-, and HOO^- Radicals and Anions

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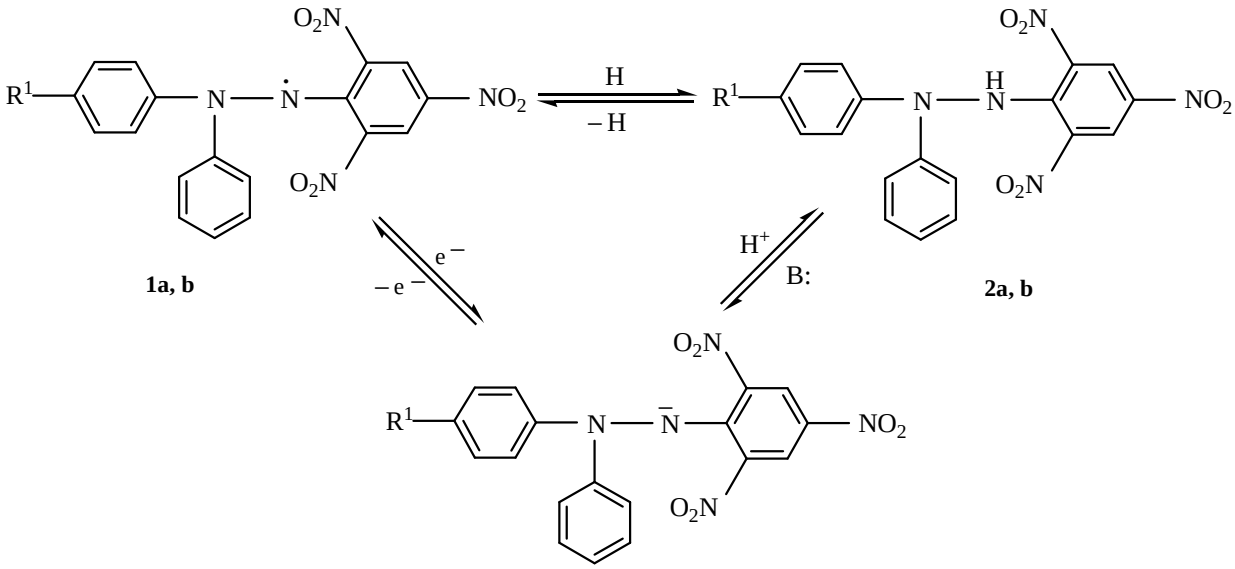
Abstract: Using electronic absorption spectra and thin layer chromatography, the reaction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with $O_2^{\bullet-}$, $HO^{\bullet}$, HO^-, and HOO^- anions and free radicals revealed the formation of the *para*-nitro- and *para*-hydroxy-derivatives of 2,2-diphenyl-1-picrylhydrazine (DPPH-H) and of DPPH fragmentation products (diphenylamine, tetraphenylhydrazine). The reaction of DPPH with the $O_2^{\bullet-}$ anion-radical (from KO_2 in benzene solution at room temperature in the presence of 18-crown-6 ether) is pseudo-first-order during the first 25 minutes.

Keywords: Diphenylpicrylhydrazyl; Reactions with potassium superoxide, hydrogen peroxide, hydroxy free radical.

1. Introduction

The free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) **1a** [1-3] has found many applications due to its high stability and intense purple color that changes whenever it reacts [4-11]. Its reduction affords 2,2-diphenyl-1-picrylhydrazine (DPPH-H) **2a**, or the corresponding anion, **3a** ($DPPH^-$) in basic medium, as shown in Scheme 1. The DPPH radical acts as a scavenger for other odd-electron species which afford *para*-substitution products at phenyl rings: thus NO_2 yields the mono-nitro-DPPH-H (**2b**) or a dinitro-DPPH-H [12-15]; the hydroxy free radical HO affords the hydroxy-DPPH-H (**7**) or its betainic oxidation product **8** [16]; halogen atoms can also be trapped similarly [4,13,14]. Other reactions that have been observed involve polynitroaniline [17,18], *ipso*-substitution of a nitro group of the picryl moiety, and *meta*-attack on this group [19].

Scheme 1. Redox and protolytic reactions for compounds **1**, **2** and **3**.



a) $R^1 = H$ (namely DPPH for **1a**; DPPH-H for **2a**; $DPPH^-$ for **3a**)

b) $R^1 = NO_2$ (namely O_2NDPPH for **1b**; O_2NDPPH-H for **2b**; O_2NDPPH^- for **3b**)

The biochemical process known as *oxidative stress* involves chemical species presented in eq. 1 [20-22].

$$O_2 \xrightarrow{e^-} O_2^{\bullet -} \xrightarrow{e^- (+2H^+)} H_2O_2 \xrightarrow{e^-} HO^{\bullet} (+ HO^-) \xrightarrow{e^- (+H^+)} H_2O \qquad (1)$$

In the oxidative stress syndrome, H_2O_2 as well as the radicalic species $HO^{\bullet}$ and $O_2^{\bullet -}$ are supposed to play an important part. Enzymes such as catalase, xanthine-oxidase, peroxidase, and superoxide-dismutase convert such species into less aggressive compounds. If DPPH (**1a**) may be used for monitoring some of the above reactions as indicated in the literature [11, 16, 23], a study of each reaction should bring interesting information. Depending on the pH, the anions may interact with DPPH *via* redox processes (electron transfer) [8, 11, 15, 19, 23-27].

For exploring the possibility of using colorimetric methods in monitoring such processes, we investigated the reaction of DPPH with the $HO^{\bullet}$ radical, $O_2^{\bullet-}$ radical-anion, HO^-, and HOO^- anions using qualitative and densitometric thin-layer chromatography (TLC) for detecting and separating the reaction products, and spectrophotometric methods for following the reaction kinetics.

2. Results and discussion

All investigations were carried out in the same solvent (benzene), either in homogeneous or heterogeneous—liquid/liquid (ℓ/ℓ) or solid/liquid systems (s/ℓ). Spectral methods were used directly with the mixture of reaction products in the benzene solution, whereas TLC separations were carried out after extracting the benzene solution with 1N hydrochloric acid.

The reactions of DPPH with the three anions ($O_2^{\bullet-}$, HO^-, and HOO^-) will be reported sequentially.

2.1. Reaction with potassium superoxide in the presence of crown ether 18C6

The $O_2^{\bullet-}$ radical-anion [28-33] may act either as reducing or oxidizing agent in the presence of copper-containing enzymes such as superoxide-dismutase. Under the influence of this enzyme the radical anion undergoes a dismutation into O_2 and H_2O_2 (eqs. 2 and 3).

$$Enz–Cu^{II} + O_2^{\bullet-} \rightarrow Enz–Cu^{I} + O_2 \quad (2)$$

$$Enz–Cu^{I} + O_2^{\bullet-} + 2H^+ \rightarrow Enz–Cu^{II} + H_2O_2 \quad (3)$$

On using commercial potassium superoxide ($K^+ O_2^{\bullet-}$) [31] in the presence of crown ether 18C6 one obtains a homogeneous benzene solution in which the nucleophilic reactivity of the anion is enhanced [30, 34-37]. Preliminary investigation revealed that the intensity of the characteristic absorption band of DPPH (**1a**) at λ_{max} = 520 nm decreases due both to the formation of the anion (**3a**) with λ_{max} = 428 nm [8, 38] *via* a redox process involving the superoxide radical-anion (eqs. 4 and 5), and to other reactions. In all equations, subscripts $_s$, $_o$ and $_w$ denote solid, organic (benzene) and, aqueous phases, respectively.

$$KO_{2\,(s)} + 18C6_{\,(o)} \rightleftharpoons [18C6…K]^+ O_2^{\bullet-}{}_{(o)} \quad (4)$$

$$[18C6…K]^+ O_2^{\bullet-}{}_{(o)} + DPPH_{\,(o)} \rightarrow [18C6…K]^+DPPH^-{}_{(o)} + O_2^- \quad (5)$$

A kinetic study was performed. Determinations were carried out at 25ºC with benzene solutions of DPPH and 18C6, and solid KO_2 in molar ratios **1a**:18C6:KO_2 that were 1:1:3, 1:5:5, and 1:5:9 by monitoring the DPPH λ_{max} = 520 nm band. An excess of KO_2 (hence also of 18C6) is needed. After 24 h there is no further decrease of **1a** absorption (Table 1) and the concentration of the anion **3a** reaches a plateau.

The spectrophotometric study revealed two isosbestic points at 344 and 496 nm (Fig.1).

During the first 25 minutes, the reaction is pseudo-first-order, as seen from Fig. 2. Compounds **2b**, **4**, and **6** appear after about 30 minutes.

Table 1. Kinetics of unreacted DPPH (**1a**) in the reaction with 18C6 and KO_2 (molar ratio = 1 : 5 : 5).

Time (min)	Unreacted DPPH (%)
5	95.66
25	93.59
45	91.94
65	90.70
90	87.40
120	83.05
1440 (24h)	71.90

Figure 1. Monitoring by UV-VIS in benzene the formation of **3a** (λ_{max} = 428 nm) from **1a** (λ_{max} = 520 nm) as described by eqs. 4 and 5 (for molar ratio **1a** : 18C6 : KO_2 = 1:5:5).

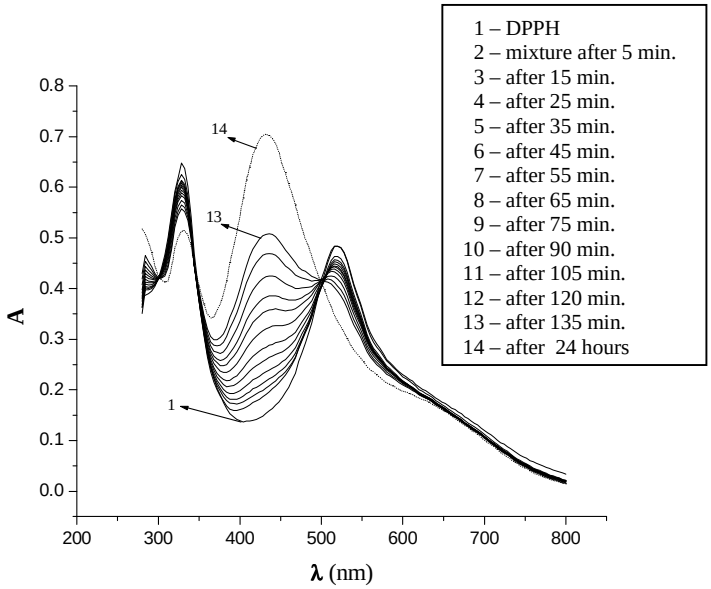
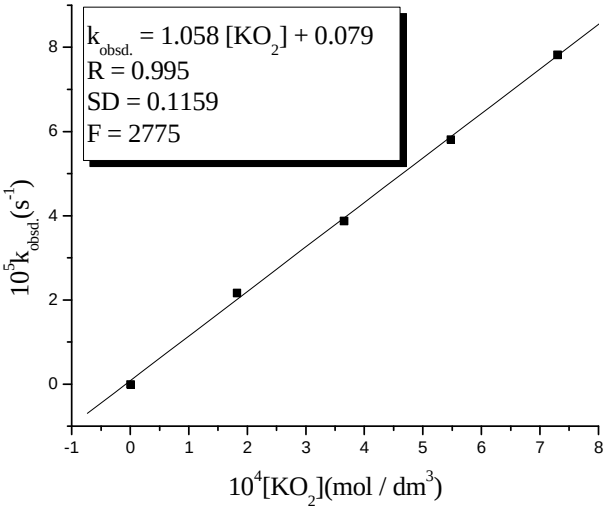
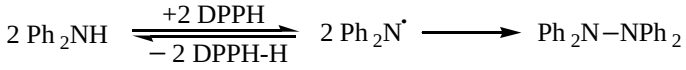


Figure 2. Pseudo-first order kinetics of the reaction between DPPH and KO_2 in benzene in the presence of 18C6 at 25ºC (the first 25 minutes).



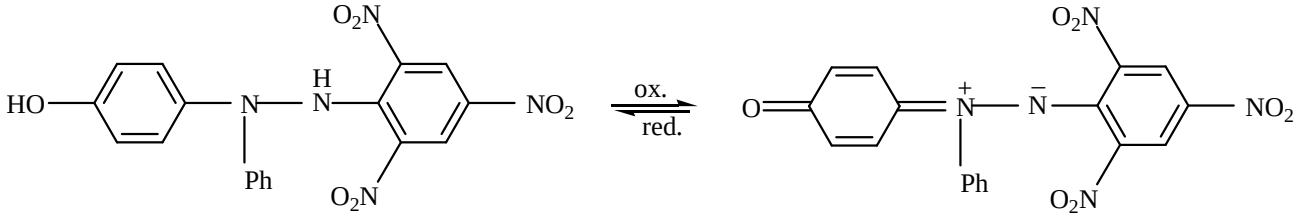
The reaction (eqs. 4 and 5) was also investigated by qualitative and densitometric TLC, allowing the observation of secondary products that are not detected by spectrophotometry. During the first 30 minutes, secondary products are not detectable either in the crude reaction mixture or after acid extraction. However, after 2 h the formation of mononitro-DPPH-H (**2b**) and of diphenylamine (**4**) could be observed, and after 24 h also tetraphenylhydrazine (**6**) was found. Neither the bis-(*p*-nitro)-DPPH-H (**3b**) nor the *p*-hydroxy- DPPH-H (**7**) and/or its oxidized betainic form (**8**) could be detected. The identity of these two products was certified by comparing the R_f values in TLC and the NMR spectra (^1H and ^{13}C) with those of authentic compounds.

Scheme 2. Redox process involving diphenylamine (**4**) and tetraphenylhydrazine (**6**).



4 **5** **6**

Scheme 3. Redox process involving 4-hydroxy- DPPH-H (**7**) and the betainic oxidized product (**8**).



7 **8**

In Table 2 we present the products that have been identified by quantitative (densitometric) TLC analysis after 24 h at room temperature by treating DPPH (**1a**) with: HO^- (from

benzyltrimethylammonium hydroxide, *reaction A*); with $HO^{\bullet}$ (from the photochemical reaction of *ortho*-hydroxy-acetophenoneoxime also called FotoFenton^{TM}2, *reaction B*), with $O_2^{\bullet -}$ (from potassium superoxide, *reaction C*), and with HOO^- (from hydrogen peroxide, *reaction D*).

Table 2. Quantitative TLC data on the reactions of DPPH (**1a**) with HO^-, $HO^{\bullet}$, $O_2^{\bullet -}$ and HOO^- in benzene after 24 h.

Reaction [c]	DPPH-H [b] **2a**		O_2N-DPPH-H[b] **2b**		Ph_2NH[a] **4**		Ph_2N-NPh_2[a] **6**		HO-DPPH-H [b] **7**		Betaine[b] **8**	
	R_f	%	R_f	%	R_f	%	R_f	%	R_f	%	R_f	%
A (HO^-)	0.67	46	–	–	0.44	22	0.68	1	0.59	8	0.04	6
B ($HO^{\bullet}$)	0.68	20	0.31	12	0.44	25	–	–	0.57	11	0.04	9
C ($O_2^{\bullet -}$)	0.67	65	0.30	12	0.43	4	0.67	0.4	–	–	–	–
D (HOO^-)	0.67	70	0.29	10	0.44	3	–	–	–	–	0.03	5

[a,b] TLC on silica gel: [a] without acid extraction using as eluent n-hexane : toluene = 7 : 3 (v/v); [b] after extraction with 1N hydrochloric acid using toluene as eluent.

[c] *Reaction A*: **1a** + benzyltrimethylammonium hydroxide (molar ratio 1:2); *Reaction B*: **1a** + hν + hydroxy-acetophenoneoxime (molar ratio 1:2); *Reaction C*: **1a** + 18C6 + KO_2 (molar ratio 1:5:5); *Reaction D*: **1a** + Kryptofix 222 + H_2O_2 (molar ratio 1:2:2).

The formation of $DPPH^-$ anion (**3a**) and redox processes may explain the production of the observed products, as indicated by eqs. (6) – (9).

$$[18C6...K]^+DPPH^-_{(o)} + O_2^{\bullet -}{}_{(o)} \rightleftharpoons DPPH_{(o)} + [18C6...K]^+O_2^{2-}{}_{(o)} \quad (6)$$

$$DPPH^-_{(o)} + 2\ O_2^{\bullet -}{}_{(o)} \rightleftharpoons [DPPH^-, 2\ O_2^{\bullet -}]_{(o)} \rightarrow NO_2^-{}_{(o)} + PRODUCTS_{(o)} \quad (7)$$

$$NO_2^-{}_{(o)} + DPPH_{(o)} \rightarrow DPPH^-_{(o)} + NO_2^{\bullet}{}_{(o)} \quad (8)$$

$$DPPH_{(o)} + NO_2^{\bullet}{}_{(o)} \rightarrow O_2NDPPH\text{-}H_{(o)} \quad (9)$$

One may speculate that DPPH (**1a**) may split into the diphenylamino radical (**5**) that dimerizes [1, 39, 40] to tetraphenylhydrazine (**6**), and dinitrobenzofuroxan (from picrylnitrene), but as yet there is no experimental evidence for this conjecture. However, we intend to look for such evidence among the many side products detected by TLC. Also, σ-Meisenheimer complexes (eq. 7) may be involved in the formation of some of the observed reaction products [23, 24, 41].

2.2. Reaction of DPPH with the hydroxide anion and the hydroxy free radical

Previous papers reported that on treating DPPH (**1a**) with hydroxide anions (from powdered alkali hydroxides in the presence of crown ethers or polyethylene glycol) a redox process takes place

affording hydroxyl free radicals ($HO^{\bullet}$) and resulting in the formation of 4-nitro-DPPH-H (**2b**) and 4-hydroxy-DPPH-H (**7**) [25, 42]. Also the reaction between DPPH and photolytically-generated $HO^{\bullet}$ [43] radicals was investigated, leading to 4-hydroxy-DPPH-H (**7**) and its betainic oxidized product (**8**), which were isolated [16].

The present paper reports results for the reaction between DPPH and $HO^{\bullet}$ radicals generated by three methods: (i) photochemically, (ii) from solid potassium hydroxide, DPPH, and 18C6, and (iii) from DPPH, 18C6, and benzyltrimethylammonium hydroxide.

(i) As shown in Table 2, in the photochemical reaction, in addition to compounds **2b**, **7**, and **8**, diphenylamine (**4**) was also identified and determined quantitatively.

(ii) From the reaction with solid potassium hydroxide the same four products were detected qualitatively.

(iii) The reaction with benzyltrimethylammonium hydroxide afforded **2b**, **6**, **7**, and tetraphenylhydrazine (**4**).

Eqs. (7 – 9) and (12) make plausible the formation of $NO_2^{\bullet}$ radicals, which could explain the formation of **2b** from DPPH. Then eqs. (10 – 14) could explain the formation of compounds **4** and **6** (along perhaps with dinitro-benzofuroxan among the products).

$$DPPH_{(o)} + [18C6...K]^{+}HO^{-}_{(o)} \longrightarrow [18C6...K]^{+}DPPH^{-}_{(o)} + HO^{\bullet}_{(o)} \quad (10)$$

$$DPPH_{(o)} + HO^{\bullet}_{(o)} \longrightarrow \mathbf{7}_{(o)} \quad (11)$$

$$DPPH_{(o)} + HO^{\bullet}_{(o)} \longrightarrow NO_2^{\bullet} + PRODUCTS_{(o)} \quad (12)$$

$$DPPH^{-}_{(o)} + 2\,HO^{-}_{(o)} \rightleftharpoons [DPPH^{-}, 2\,HO^{-}]_{(o)} \quad (13)$$

$$[DPPH^{-}, 2\,HO^{-}]_{(o)} \longrightarrow \mathbf{4}_{(o)} + \mathbf{6}_{(o)} + PRODUCTS_{(o)} \quad (14)$$

2.3. Reaction of DPPH with the hydrogen peroxide anion

As discussed above, eq. 1 indicates that hydrogen peroxide is formed in living cells, and rapidly decomposed by the defense enzymatic system (catalase, peroxidase). Numerous reagents may be used for assaying hydrogen peroxide and derived oxidizing species in organisms [43]. From the reaction of DPPH with the Fenton reagent (hydrogen peroxide and ferrous sulfate, which afford hydroxy free radicals $HO^{\bullet}$), quantitative TLC determinations certified the formation of mononitro- and monohydroxy-DPPH-H derivatives **2b** and **7**, respectively [16].

Now we report the reaction of DPPH (**1a**) with the HOO^{-} anion formed from hydrogen peroxide and the basic compound Kryptofix 222 (*4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8] hexacosane*), abridged here as K_{222}, (eq. 15) [44]. The reaction was carried out with DPPH and K_{222} (molar ratios 1:2) with an excess of 30% H_2O_2 in a liquid/liquid biphasic system (water/benzene) at room temperature under stirring. During the first three hours the process was monitored by qualitative TLC, and after 48 h by quantitative TLC. The mono-nitro derivative **2b** and the betaine **8** were identified after 3 h, implying that the mono-hydroxy derivative **7** must have been formed initially (Scheme 3). After 24 h, in addition to **2b** and **8**, the presence of diphenylamine (**4**) was detected, and all these three products were assayed quantitatively (Table 2). These results may be explained as being due to attack on DPPH by the $HOO^{\bullet}$ / $HO^{\bullet}$ radicals or the HOO^{-} / HO^{-} anions formed by K_{222}, the

latter being basic enough to react with hydrogen peroxide or water and the corresponding anions then becoming oxidized by DPPH (eqs. 15 – 18). Finally, the reactions between DPPH and the free radicals afford the observed products (eqs. 11 – 14 and 19 – 20) [44].

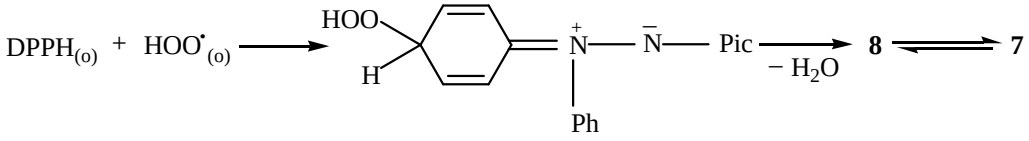
$$K_{222\,(o)} + H_2O_{2\,(w)} \rightleftharpoons [K_{222}\,H]^+HOO^-_{(o)} \quad (15)$$

$$[K_{222}\,H]^+HOO^-_{(o)} + DPPH_{(o)} \longrightarrow [K_{222}\,H]^+DPPH^-_{(o)} + HOO^{\bullet}_{(o)} \quad (16)$$

$$K_{222\,(o)} + H_2O_{(w)} \rightleftharpoons [K_{222}\,H]^+HO^-_{(o)} \quad (17)$$

$$[K_{222}\,H]^+HO^-_{(o)} + DPPH_{(o)} \longrightarrow [K_{222}\,H]^+DPPH^-_{(o)} + HO^{\bullet}_{(o)} \quad (18)$$

$$DPPH^-_{(o)} + 2\,HOO^-_{(o)} \rightleftharpoons [DPPH^-,\,2\,HOO^-]_{(o)} \longrightarrow PRODUCTS_{(o)} \quad (19)$$



(20)

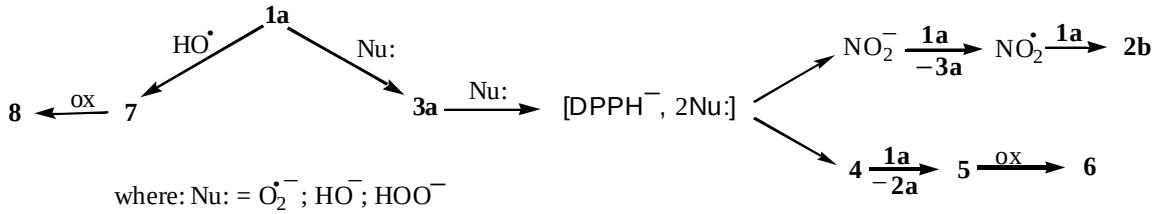
2.4. Comparison between reactions of DPPH with $O_2^{\bullet-}$, $HO^{\bullet}$, HO^-, and HOO^-

On investigating the room-temperature reaction of the stable free radical DPPH with oxidizing species $O_2^{\bullet-}$, $HO^{\bullet}$, and HOO^- that appear in biological processes, it was found that in all cases the electronic absorption spectra could provide information during the first 25 minutes, but TLC methods were more adequate for monitoring the reaction at longer times, when various products interfered with the spectral determinations.

The concentration decrease of the starting material is highest for HOO^- and lowest for $HO^{\bullet}$, leading to the following reaction rate order: $HOO^- > O_2^{\bullet-} > HO^- > HO^{\bullet}$. Excepting the reaction *A* with hydroxide anions, the mono-nitro-DPPH-H compound (**2b**) was formed in comparable concentrations in all experiments (Table 2). Diphenylamine (**4**) was formed in different amounts: $HO^{\bullet} \approx HO^- > O_2^{\bullet-} \approx HOO^-$, and the derived tetraphenylhydrazine (**6**) was identified only in very small concentrations in the reactions with $O_2^{\bullet-}$ and HO^-. The mono-hydroxy-DPPH-H compound (**7**) and its betainic oxidized derivative (**8**) were detected in low amounts in all reactions except for reaction *C* with $O_2^{\bullet-}$. It may be possible that all products were always formed, but in some cases at lower amounts than could be detected by TLC techniques.

A mechanism involving σ-Meisenheimer complexes of DPPH with the hydride anion was proposed earlier [41]. In Scheme 4 we propose two distinct mechanisms for the reactions reported in the present paper. On the left-hand side, the homolytic attack of the hydroxyl radical $HO^{\bullet}$ explains the formation of mono-hydroxy-DPPH-H (**7**) and its oxidized betainic derivative (**8**). A different pathway for the formation of compounds **7** and **8** is suggested by eq. 20. On the right-hand side the nucleophilic attack of anions Nu: (HOO^- and HO^- or radical-anion $O_2^{\bullet-}$) may lead to the formation [16, 23] of an unstable σ-Meisenheimer complex [$DPPH^-$, 2Nu:]. In turn, this complex is supposed to cleave either a nitro group explaining the formation of the mono-nitro-DPPH-H (**2a**), or (supposedly) dinitro-benzofuroxan and diphenylamine (**4**), which is then further oxidized to tetraphenylhydrazine (**6**).

Scheme 4. Mechanisms proposed for the reactions of DPPH with $HO^{\bullet}$ free radicals and nucleophilic agents (Nu:).



Taking into account that DPPH is able to abstract a hydrogen atom from amines with a significant reaction rate, as determined by K. U. Ingold and his coworkers [45], even more complicated mechanisms may be envisaged, in addition to the process converting **4** into **6**.

3. Conclusions

Several conclusions can be drawn from the study of reactions between DPPH and anions or oxidizing species: $O_2^{\bullet -}$, HO^-, and HOO^-. Redox processes involving DPPH convert such anions into oxidants ($HO^{\bullet}$, $HOO^{\bullet}$). The formation of minor amounts of reaction products can be detected well by TLC, but less well (and only during the first half-hour at room temperature) by spectrophotometric techniques. The following reactions products were detected and assayed quantitatively: DPPH-H (**2a**), its mono-nitro- (**2b**) and mono-hydroxy-derivatives (**7**), the betainic oxidized form (**8**) of the latter, diphenylamine (**4**) and tetraphenylhydrazine (**6**) resulted by cleavage and oxidation of DPPH. The reaction mechanisms are fairly complicated, involving protolytic equilibria and redox processes, accompanied by molecular fragmentations.

4. Experimental part

Commercial chemicals were purchased from Aldrich (DPPH and KO_2), Merck (18C6, Kryptofix-222, diphenylamine, benzyltrimethylammonium hydroxide, glass plates with silica gel GF_{254} silanized and nonsilanized), and Molecular Probes (*ortho*-hydroxy-acetophenoneoxime – FotoFenton^{TM}2). The mono-nitro-DPPH-H (**2b**) [46], the solid supramolecular complex [18C6…K]$^+$DPPH$^-$ [47], and tetraphenylhydrazine (**6**) [39], were prepared according to literature data.

Electronic absorption spectra were recorded with a Unicam-UV-VIS spectrophotometer using "Vision Software V.3.33". NMR spectra were recorded with a Varian Gemini 300 BB spectrometer (300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR) using TMS as internal standard. We used Camag Software 1992 scanner II – Switzerland for densitometric TLC analysis.

Reactions of 2,2-diphenyl-1-picrylhydrazyl, DPPH (**1a**) *with KO_2, KOH, benzyltrimethylammonium hydroxide, FotoFenton^{TM}2, and H_2O_2 at room temperature.*

A1. *The reaction with hydroxide anions from solid KOH in the presence of 18C6* was carried out in benzene for 2 hrs under stirring with molar ratio **1a**:18C6:KOH = 1:2:2. After liquid/liquid (ℓ/ℓ) extraction with 1N hydrochloric acid, the separated organic layer was dried with anhydrous sodium

sulfate and analyzed by TLC (silica gel, toluene). For the quantitative TLC analysis the chromatograms were scanned at λ_{max} = 254 nm, and the densitometric results are presented in Table 2.

A2. *The reaction with hydroxide anions from benzyltrimethyl ammonium hydroxide* was carried out in benzene under stirring wither for 2 hrs or 48 hrs with molar ratio between DPPH and the quaternary ammonium salt 1:2. Without ℓ/ℓ extraction, the solution was analyzed qualitatively and quantitatively by scanning (λ_{max} = 254 nm) and results are indicated in Table 2.

B. *The reaction with hydroxy free radicals* ($HO^{\bullet}$) was performed in benzene under stirring using DPPH and *ortho*-hydroxy-acetophenoneoxime (FotoFenton^{TM}2) with ultraviolet irradiation in a quartz flask. Without ℓ/ℓ extraction, the solution was analyzed qualitatively and quantitatively by scanning (λ_{max} = 254 nm) and results are indicated in Table 2.

C. *The reaction with solid KO_2 in the presence of 18C6 was carried out in benzene under* stirring with three molar ratios of the reactants: **1a**:18C6:KO_2 = 1:1:3, 1:5:5, and 1:5:9. Spectrophotometric analysis for the decrease of DPPH concentration (λ_{max} = 520 nm) and the increase of $DPPH^-$ concentration (λ_{max} = 428 nm) allowed us to follow the reaction kinetics (Figures 1 and 2). Product formation was analyzed by two methods: (i) without ℓ/ℓ extraction by TLC (silanized silica gel, n-hexane; and nonsilanized silica gel, n-hexane:toluene 7:3 v/v); (ii) with ℓ/ℓ extraction, as for reaction *A*.

D. *The reaction with HOO^- anions from H_2O_2 and K_{222}* was performed in a biphasic benzene-aqueous ℓ/ℓ system under stirring with molar ratio **1a**:K_{222}:H_2O_2 = 1:2:2. After 2 hrs, the organic phase was separated, dried, and without ℓ/ℓ extraction the solution was analyzed qualitatively and quantitatively by scanning (λ_{max} = 254 nm); the results are indicated in Table 2.

*Kinetics of the reaction between DPPH (**1a**) and potassium superoxide.*

For studying the kinetics of the reaction **1a** + 18C6 + KO_2, the temperature was fixed at 25ºC, and two concentrations were kept constant (**1a** and 18C6); the concentration of potassium superoxide was varied according to molar ratios **1a:**18C6:KO_2 = 1:5:2; 1:5:3; 1:5:4 and 1:5:5. The formation of the $DPPH^-$ anion (**3a**) was monitored at λ_{max} = 428 nm. For determining the order of the reaction, the averages of five results for each of the five spectral measurements were plotted as k_{obs} vs. concentration of KO_2 (Fig. 2) according to eq. 21.

$$k_{obs} = 1/t \ln[a/(a–x)] \quad (21)$$

where t is the time (seconds), a is the initial concentration of **3a** (absorbance A_0 at $t = t_0$, and $(a–x)$ is the concentration at time t.

Reaction of the supramolecular complex $[18C6…K]^+DPPH^-$ with potassium superoxide.

To the supramolecular complex $[18C6…K]^+DPPH^-$ [46] dissolved in benzene, solid KO_2 and 18C6 (molar ratio Complex:18C6:KO_2 = 1:4:5). The concentrations of **1a** (λ_{max} = 520 nm) and of the $DPPH^-$ anion **3a** (λ_{max} = 428 nm) were monitored spectrophotometrically. Qualitative TLC analysis after 2h revealed the presence of DPPH (**1a**) and diphenylamine (**4**) using silanized silica gel (n-hexane), nonsilanized silica gel (n-hexane:toluene, 7:3 v/v) and nonsilanized silica gel (toluene).

*NMR Identification of reaction products mono-nitro-DPPH-H (**2b**), diphenylamine (**4**), and tetraphenylhydrazine (**6**).*

Using reactions that afforded significant yields of products **2b**, **4**, and **6** (Table 2) and preparative conditions (starting with 0.1 g of DPPH), the reaction products were separated and purified as follows: for **2b**, the preparative TLC plate was extracted with a mixture of methylene chloride and methanol 9:1 v/v. For **4** and 6 the extractions were performed with methylene chloride and the solvent was removed under vacuum and in argon atmosphere in order to avoid oxidation by air oxygen.

*Diphenylamine **4**:* ^{1}H-NMR($CDCl_3$, δ ppm, J Hz): 5.61(bs, 1H, NH, deuterable); 6.90(tt, 2H, H-*para*, 1.1, 7.3); 7.03(dd, 4H, H-*ortho*, 1.1, 8.5); 7.23(dd, 4H, H-*meta*, 7.3, 8.5); ^{13}C-NMR($CDCl_3$, δ ppm): 143.08(C-1); 129.27(C-*meta*); 120.92(C-*para*); 117.79(C-*ortho*).

*Tetraphenylhydrazine **6 :*** ^{1}H-NMR($CDCl_3$, δ ppm, *J* Hz): 6.98(tt, 1H, H-*para*, 1.1, 7.2); 7.20(dd, 2H, H-*meta*, 7.2); 7.31(dd, 2H, H-*ortho*, 7.2, 8.2); ^{13}C-NMR($CDCl_3$, δ ppm): 142.91(C-1); 129.07(C-*meta*); 122.08(C-*para*); 118.18(C-*ortho*).

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