Indian Journal of Chemistry
Vol. 60A, July 2021, pp. 927-931

Kinetics and mechanistic studies for oxidation of N-benzylhydroxylamine by a Co^{III}-bound bridging superoxo complex in perchloric acid medium

Sekhar Gain*
Department of Chemistry, Ramakrishna Mission Vidyamandira, Howrah 711 202, West Bengal, India
*E-mail: sekhargain@gmail.com

Received 10 February 2021; revised and accepted 04 May 2021

In aqueous perchloric acid medium (pH = 0.522 – 1.3), N–benzylhydroxylamine (two electron reductant) reduces the one electron oxidant, superoxo ligand in $[(dien)(en)Co^{III}(O_2)Co^{III}(en)(dien)](ClO_4)_5$ (**1**) to the corresponding hydroperoxo complex, $[(en)(dien)Co^{III}(HO_2)Co^{III}(en)(dien)]^{5+}$ (**2**) and itself gets oxidised to $PhCH_2NO$ following both proton coupled electron transfer path and an electron transfer reaction. The kinetics, stoichiometry and reaction mechanism clearly indicate that oxidation of $PhCH_2NHOH$ occurs through the formation of an intermediate, benzyl derivative of aminoxyl radical ($PhCH_2NHO^{\bullet}$). In the presence of excess $PhCH_2NHOH$ over **1**, the reaction obeys first-order kinetics and rate of the reaction increases with $[PhCH_2NHOH]$. The reaction rate, however, decreases with increase in $[H^+]$ and the plot of $1/k_o$ with $[H^+]$ is linear with a small but noteworthy intercept. It is also remarked that the reaction rate remarkably decreases with increasing proportion of D_2O replacing H_2O in the solvent. Therefore, an H-atom transfer from $PhCH_2NHOH$ to the bridging superoxide in **1** seems reasonable at the rate determining step.

Keywords: N-benzylhydroxylamine, Superoxo, Kinetics, Mechanisms, Oxidation- reduction

In the recent time hydroxylamine and its derivative are of very important as they are the best sources of reactive nitroso (N–O bond) intermediates[1] and for the synthesis of both chiral and bioactive compounds the N-selective as well as O-selective intermediates are very important[2]. The intermediate derivatives of hydroxylamine are having much attention for their usage in the preparation of Aziridines[3], Isoxazolidinones and β-Amino acids[4]. N-benzylhydroxylamine ($PhCH_2NHOH$) is a substitution derivative of both hydroxylamine and NH_3 and it is a powerful inhibitor, have pharmacological and therapeutic effects. Indeed $PhCH_2NHOH$ is a very good useful chemical and gives better results compared with other similar compounds for the preparation of antiseptic, antibiotic and anti fungal compounds[5]. Moreover, N–O bonded compound has interesting atypical structure and special type of biological activity[6]. When hydroxyl group is protected, e.g., O-benzylhydroxylamine, the species has multidimensional pharmaceutical activity and magnifies the medical activity such as; it is chemically used for the preparation of antiseptic, antibiotic and anti fungal compounds[7]. From the literatures screening as well as available pharmaceutical resources, it is clear that, O-benzylhydroxylamine is fundamentally drug potential. In the twenty-first century the infectious diseases constitute a tenacious and major public health problem worldwide[8]. In this regards N-substituted hydroxylamine derivatives are very important because it is a very good radical scavenger and can also be used for treatment of cancer[8]. The derivative hydroxyurea is very important because it effectively inhibit the Ribonucleotide reductase (RNR) of eukaryotic cells and most commonly used as an inhibitor of the growth of gram-positive and gram-negative bacteria[9]. Moreover for the treatment of cancer and bacterial infections disease without interfering in human RNR, N-substituted hydroxylamine is very important[9]. At the same time hydroxylamine and some of its N- and O – substituted derivatives show erythrotoxic effect[10]. Oxyhemoglobin reacts with hydroxylamine and its O-derivatives leading to, Heinz body formation and red cell hemolysis[11]. In addition to these it also gives rise to radical intermediates, which cause lipid peroxidation and lead to impairment of some essential detoxification enzymes[12]. Similarly some of the N-substituted hydroxylamine inhibits the activity of glucose 6-phosphate dehydrogenase and glutathione reductase.

On the other hand all the living organisms contain superoxide and all the hydroxylamine reacts with oxyhemoglobin to produce superoxide, radical intermediate and in some cases secondary products are also formed[13]. So the chemistry of N/ O – substituted hydroxylamine and superoxide especially with

the metal bound superoxide is very important. The present work represents the kinetics and reaction mechanism of the reaction between $PhCH_2NHOH$ and a Co^{III} -coordinated superoxide ligand in perchloric acid medium.

Materials and Methods

Superoxo complex, μ-superoxo[bis(ethylenediamine) bis(diethylenetriamine)cobalt(III)]$^{5+}$, viz., [(dien)(en)$Co^{III}(O_2)Co^{III}$(en)(dien)]$(ClO_4)_5$ (**1**) was synthesized and characterised following the literature method[14,15]. $NaClO_4$ was prepared by neutralizing $HClO_4$ with $NaHCO_3$ in the usual way. All other materials including N-benzylhydroxylamine (Aldrich) and dipicolinic acid (dpa, Aldrich) were used as received. Freshly boiled, double distilled water was used to prepare all the solutions necessary for the experiments.

UV-visible spectra and changes in absorbance of the reaction mixture during the kinetic experiments were recorded with Shimadzu spectrophotometer (1800) equipped with electrically controlled thermostat (25 ± 0.1 °C) and 1 cm quartz cells. A pH meter (Gold-533) with electrodes calibrated with standard buffer solutions was used for pH measurements, C, H, N analyses were made using a 2400 series-II CHN/O analyzer (Perkin Elmer).

Complex **1** shows its characteristic absorbance at 708 nm where no other reactants absorb. In aqueous perchloric acid media the simple reaction with large excess of $PhCH_2NHOH$ ([$PhCH_2NHOH$] >> [**1**], i.e., pseudo first order condition, [$PhCH_2NHOH$] is the analytical concentration of $PhCH_2NHOH$**)**, under these conditions, the reactions obeyed excellent first-order kinetics at least up to 95% completion of reaction and the first order rate constants (k_o) were evaluated by non-linear least squares fitting of the decay of the absorbance (A_t) with time (t) data to standard first-order exponential decay equation. Furthermore, dipicolinic acid (dpa, $C_7H_5NO_4$) was added to sequester the ubiquitous metal ions (*vide infra*) present in the reaction media. When the solvent was enriched with D_2O, the pH of the reaction media was estimated using the relation, pD = pH + 0.4[16]. Ionic strength (*I*) of the media was maintained at 0.5 M by adding $NaClO_4$.

For the determination of stoichiometry, the equilibrium absorbance of a mixture of $PhCH_2NHOH$ with 4-5 times of **1** was measured after ~6 h at 708 nm and the concentration of unused **1** in such a product mixture was determined spectrophotometrically at 708 nm.

Results and Discussion

Each mole of $PhCH_2NHOH$ consumed almost entirely 2 moles (Table 1) of the superoxo complex **1**. In addition, it is also observed that the final spectrum is almost similar in shape and peak positions (Fig. 1) to those determined for the hydroperoxo analogues of complex **1**[17,18]. A simple transformation of **1** to the hydroperoxo complex **2** is therefore visualized as per Eqn (1) given below. The observed stoichiometric ratios also entrenched $PhCH_2NO$ is the oxidation product for oxidation of $PhCH_2NHOH$.

$$2[(en)(dien)Co^{III}(O_2)Co^{III}(en)(dien)]^{5+} + PhCH_2NHOH \rightarrow 2[(en)(dien)Co^{III}(\mu O_2H)Co^{III}(en)(dien)]^{5+} + PhCH_2NO \quad \ldots (1)$$

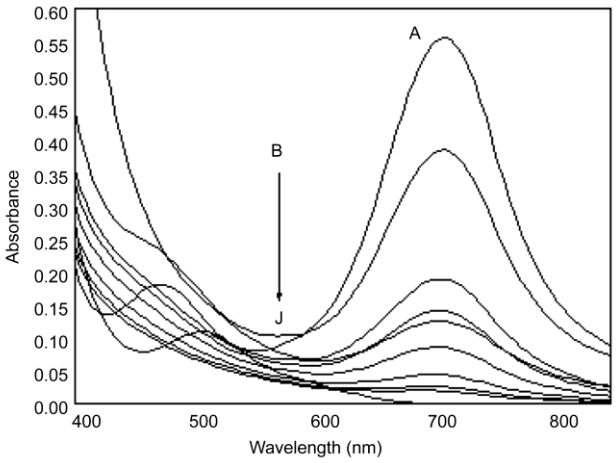


Fig. 1 — Absorption spectra of 0.50 mM of **1** reacting with 5.0 mM $PhCH_2NHOH$, (A) spectrum of pure complex , (B)-(J) spectra of reaction mixture at time intervals 60, 240, 360, 480, 600, 720, 900, 1200 and 86400 s, respectively, conditions: pH = 0.698 in perchloric acid, *I* = 0.5 M ($NaClO_4$), [dpa] = 2.0 mM, T = 25 °C

Table 1 — Stoichiometric results for oxidation of $PhCH_2NHOH$ by complex **1**, *I* = 0.5 M ($NaClO_4$). T = 25 °C, [a]pH = 1.3, [b]pH = 1.0, [c]pH = 0.698

[**1**] (mM)	[$PhCH_2NHOH$] (mM)	$[\mathbf{1}]_{left}$ (mM)	Δ[**1**] / Δ[$PhCH_2NHOH$]
13.5	3.50	6.6	1.97[a]
15.0	4.50	6.1	1.98[b]
25.0	6.0	13.1	1.98[c]

Table 2 — Variation of k_o with [$PhCH_2NHOH$], [**1**] = 0.50 mM, (a) pH = 1.0 and (b) pH = 0.698, [dpa] = 2.0 mM, I = 0.5 M, T = 25 °C

[$PhCH_2NHOH$] (mM)	5.0	10.0	15.0	20.0	25.0	30.0
(a) k_{obs} (s) x 10^3	1.5	3.1	4.9	6.6	8.5	10.8
(b) k_{obs} (s) x 10^3	1.01	2.1	3.1	3.95	5.6	6.4

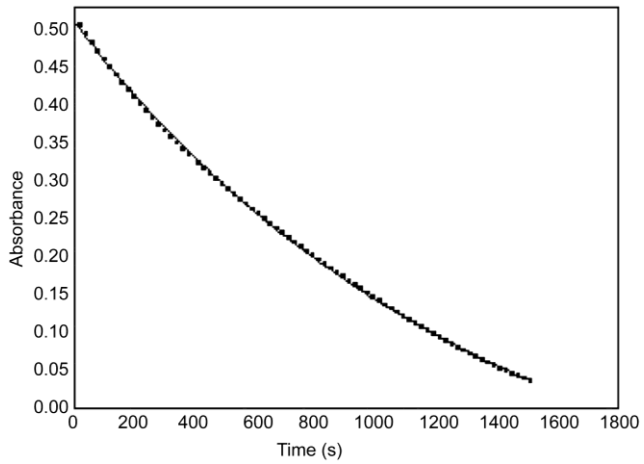


Fig. 2 — Decrease in absorbance (points shown in black circles) of **1** with time at 708 nm in its reaction with $PhCH_2NHOH$ gives an excellent fit (solid line) to the first-order exponential decay equation, conditions: [**1**] = 0.50 mM, [$PhCH_2NHOH$] = 5.0 mM, pH =. 698, I = 0.5 M ($NaClO_4$), [dpa] = 2.0 mM, T = 25 °C

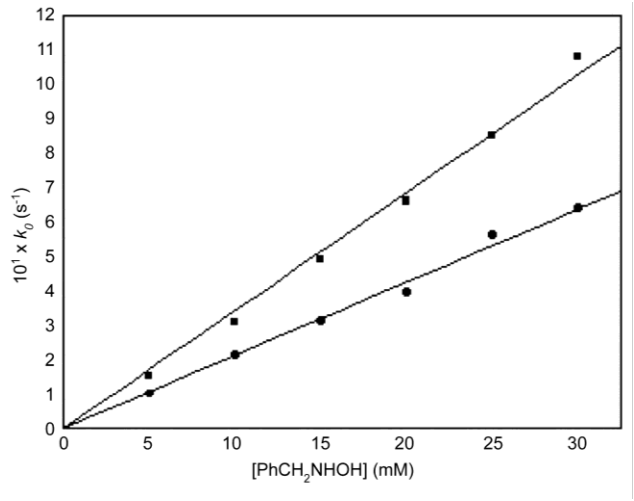


Fig. 3 — Linear variation of k_o for the reaction of [$PhCH_2NHOH$] with 1 (0.50 mM), at pH = 1.0 (■) and 0.698(●), [dpa] = 2.0 mM, I = 0.5M ($NaClO_4$), T = 25 °C

Hydroxylamine (NH_2OH) is a well known reductant and depending upon the exact reaction condition the oxidation products may vary[18]. When the oxidation is start off by a one electron oxidant or a H-atom abstractor, an intermédiate of the oxidation number N (0) is formed, which may be $H_2NO^{\bullet}$ (Aminoxyl radical) or its isomer, $^{\bullet}NHOH$. Several quantum mechanical calculation and electron

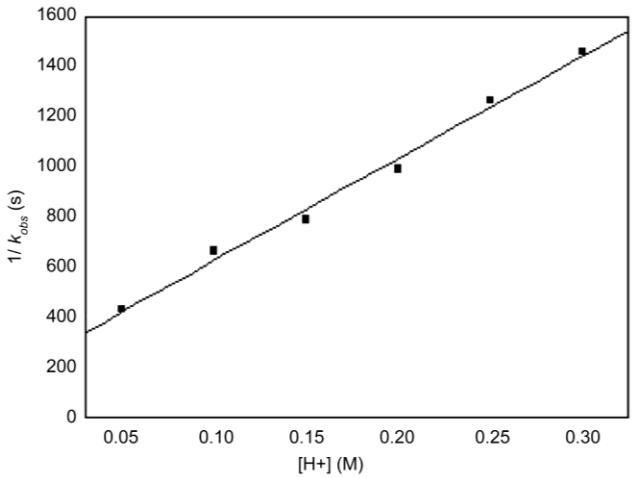


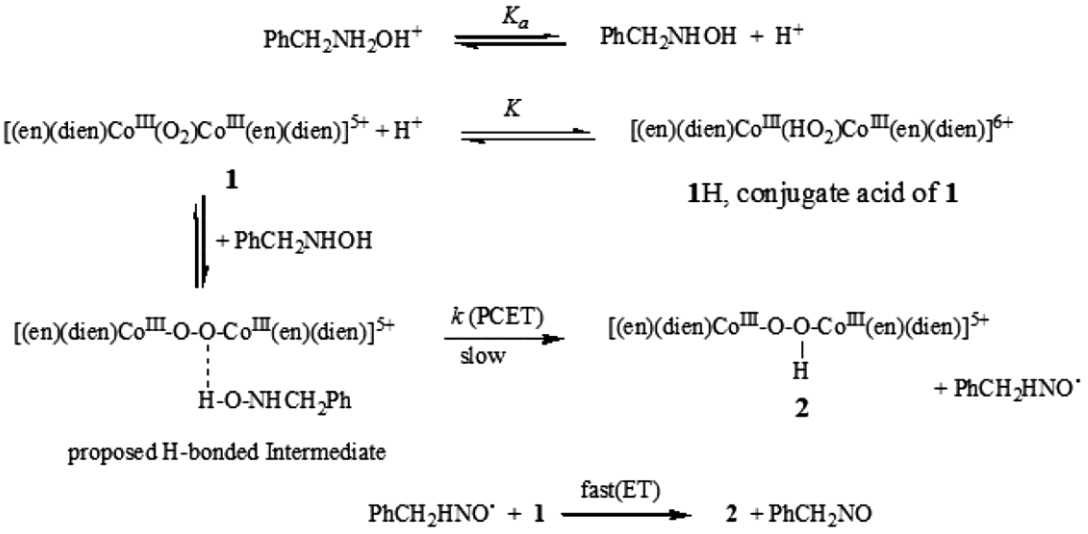
Fig. 4 — Plot of $1/k_o$ *vs* [H^+], [**1**] = 0.50 mM, [$PhCH_2NHOH$] = 5.0 mM, I = 0.5 M ($NaClO_4$), [dpa] = 2.0 mM, T = 25 °C

paramagnetic resonance (EPR) investigation predict that $NH_2O^{\bullet}$ is thermodynamically more stable than $^{\bullet}NHOH$[19]. However formation of nitroxyl radial was also reported by Zhang and Liu (2000), when mono and di-N- substituted hydroxyl amine is oxidised by a metal ion, such as neptunium(VI)[20]. Therefore, it is assumed and later on established that the product of one electron oxidation (or H-atom abstraction, proton coupled electron transfer, PCET path) of $PhCH_2NHOH$, is the benzyl derivative of aminoxyl radical ($PhCH_2HNO^{\bullet}$). $PhCH_2HNO^{\bullet}$ is a weak acid, which immediatly and very fast react with the second mole of superoxo complex **1** in an electron transfer reaction to yield benzyl derivative of nitroxyl ($PhCH_2NO$) (following electron transfer, ET path)

In aqueous perchloric acid media, complex **1** suffers no reasonable drop in absorbance over a long period of time indicating its stability against auto-decomposition. Excess N-benzylhydroxylamine, however consumes **1** and the peak absorbance (at 708 nm) drops gradually essentially to zero. The decay process followed very good first-order reaction kinetics (Fig. 2). The first-order rate constants (k_o) increased linearly with [$PhCH_2NHOH$] (Fig. 3, Table 2). The rate of the reaction were found to be greatly influenced by the acidity of reactions media and a plot of $1/k_o$ *vs* [H^+] is linear with a small but remarkable intercept (Fig. 4, Table 3). But the ionic

Table 3 — Variation of k_o with pH of the medium, [**1**] = 0.50 mM, [$PhCH_2NHOH$] = 5.0 mM, [dpa] = 2.0, I = 0.5 M, T = 25 °C

pH	1.30	1.00	0.824	0.698	0.602	0.522
1/ k_{obs}, (s^{-1})	430.5	665.02	790.50	992.47	1266.94	1460.40



Scheme 1 — Reaction mechanism

strength of the reaction media have no effect on reaction rate.

The amplification of reaction rate with pH seems not obligated from deprotonation of $PhCH_2NHOH$ as the species is weak acids (pK_a = 13.19 ± 0.3)[21] and our experimental pH range is 0.52 – 1.3. Rather, a mechanism transferring hydrogen atom (or H^+ + e) to the coordinated superoxide (hydrogen atom transfer, HAT) appears reasonable as superoxide is well-known to be a fairly strong base[22]. The observed proton-dependence on rate clearly establishes **1**H as a kinetically dead-end species. Increased proton concentration consumes more **1** from the solution forming more **1**H and consequently reaction rate falls. **1**H, being already protonated species of **1** is a redox dead-end as it has no more room to accommodate a further proton following a HAT from the reducing species. The decrease of reaction rate with [H^+] is also most likely due to the protonation of $PhCH_2NHOH$ ($PhCH_2NHOH \xrightleftharpoons{H^+} PhCH_2NH_2OH^+$). In the rate determining step, **1** is reduced to its corresponding hydroperoxo complex (**2**). Hence the mechanism can be proposed by the Scheme 1**,** shown above.

When the solvent H_2O is enriched with D_2O, there observed a significant retardation in k_o values (k_{H_2O}/k_{D_2O} ~ 2.0*)*. Moreover, the plots of k_o versus mole% of D_2O in the solvent media is found to be

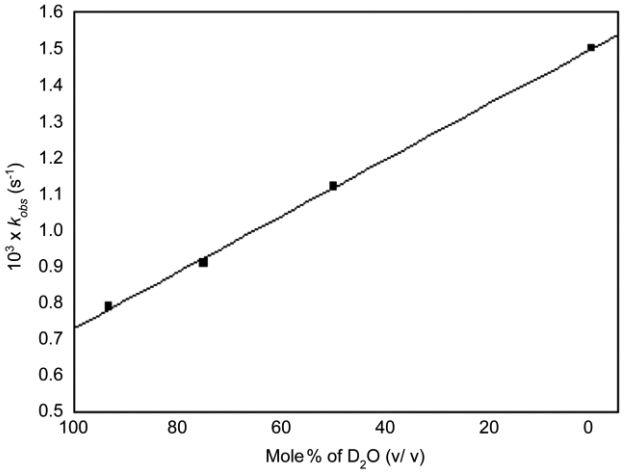


Fig. 5 — Effect of D_2O on k_o, [**1**] = 0.50 mM, [$PhCH_2NHOH$] = 5.0 mM, pD = pH + 0.4, I = 0.5 M ($NaClO_4$), [dpa] = 2.0 mM, T = 25 °C

linear (Fig. 5) indicating transfer of a single proton at the rate determining step[23]. This supports an electroprotic HAT mechanism (H^+ + e). Proposed reaction scheme in abbreviated form is as follows:

$$\mathbf{1} + H^+ \xrightleftharpoons{K} \mathbf{1}H \quad \ldots (2)$$

$$\mathbf{1} + PhCH_2NHOH \xrightarrow{k} \mathbf{2} + PhCH_2HNO^{\bullet} \quad \ldots (3)$$

Eqns (2) and (3) lead to the rate Eqn (4).

$$k_0 = k[PhCH_2NOH]/(1 + K[H^+]) \quad \ldots (4)$$

Eqn (4) may be rearranged to Eqn (5) as follows.

$$1/k_0 = 1/(k[PhCH_2NHOH] + K[H^+]/(k[PhCH_2NHOH]) \quad \ldots (5)$$

A plot of $1/k_o$ *versus* $[H^+]$ was found to be excellent straight line (Fig. 4) as expected from Eqn (5) and yielded $k = 0.91 \pm 0.036\ s^{-1}$ and $K = 3.74 (\pm 0.3) \times 10^3\ M^{-1}$. Free superoxide is a strong base ($pK_b = 9.12$)[24] and the presence of a residual basicity in a coordinated superoxide ligand is not unexpected but the basicity of the superoxide ligand due to coordination to two Co(III) centers in **1** is expectedly somewhat reduced. Again hydrogen atom transfer mechanism is an established phenomenon for phenols as reducing agents[25]. To verify the proposed mechanism, **1** was reacted with phenol and N,N-di-methyl hydroxyl amine. Both reacted with **1** but neither phenyl methyl ether nor O-methyl hydroxylamine reacted under comparable conditions and this clearly substantiates the mechanistic proposal that the presence of O–H bond is absolutely essential in the reducing agent for the reaction to proceed.

Conclusions

The two electron reductant, N-benzyl hydroxylamine reduces the one electron oxidant, superoxo ligand in $[(dien)(en)Co^{III}(O_2)Co^{III}(en)(dien)](ClO_4)_5$ (**1**) to the corresponding hydroperoxo complex, $[(en)(dien)Co^{III}(HO_2)Co^{III}(en)(dien)]^{5+}$ (**2**) following both proton coupled electron transfer) path and an electron transfer reaction. After detailed studies of kinetics, stoichiometry and reaction mechanism, it is conclde that the oxidation of $PhCH_2NHOH$ occurs through the formation of an intermediate benzyl derivative of aminoxyl ($PhCH_2NHO^{\bullet}$) radical.

Acknowledgement

This work was carried out under the financial assistance from MRP (UGC) (Sanction No: PSW-067/14-15(ERO)) and the Department of Chemistry, RKM Vidyamandira, is thankfully acknowledged.

References

1 Baidya M & Yamamoto H, *Synthesis*, 45 (2013) 1931.
2 Yamamoto Y, Momiyama N & Yamamoto H, *J Am Chem Soc*, 126 (2004) 5962.
3 Bongini A, Cardillo G, Gentilucci L & Tomasini C, *J Org Chem*, 62 (1997) 9148.
4 Pettersen D, Marcolini M, Bernardi L, Fini F, Herrera R P, Sgarzani V & Ricci A, *J Org Chem*, 71 (2006) 6269.
5 Nikulin A, Stern P & Zeger-Vidovic Z, *Arch Znt Pharmacodyn*, 166 (1967) 305.
6 Blazewska K & Gajda T, *Tetrahedron*, 59 (2003) 10249.
7 Knight D W & Leese M P, *Tetrahedron Lett*, 42 (2001) 2593.
8 Nohl H & Stolze K, *Free Radical Biol Med*, 15 (1993) 257.
9 Misra H P, *J Biol Chem*, 254 (1979) 11623.
10 Evelo C T A, Spooren A A M G, Bisschops R A G, Baars L G M & Neis J M, *Blood Cells Mol Dis*, 24 (1998) 280.
11 Spooren A A M G & Evelo C T A, *Blood Cells Mol Dis*, 23 (1997) 323.
12 Ighodaro O M & Akinloye O A, *Alexandria J Med*, 54 (2018) 287.
13 Spooren A A M G & Chris T A, *Blood Cells Mol Dis*, 26 (2000) 373.
14 Duffy D L, House D A & Weil J A, *J Inorg Nucl Chem*, 31 (1969) 2053.
15 Gain S, Mukhopadhyay S & Banerjee R, *Indian J Chem*, 51A (2012) 949.
16 Hung M D & Stanbury M, *Inorg Chem*, 44 (2005) 3541.
17 Hoffman A B & Taube H, *Inorg Chem*, 7 (1968) 1971.
18 Gain S, Mishra R, Mukhopadhyay S & Banerjee R, *Inorg Chim Atca*, 373 (2011) 311.
19 Barge J N & Gkavi G S, *Oriental J Chem*, 33 (2017) 2573.
20 Zhang A & Liu Y, *J Radioanal Nucl Chem*, 245 (2000) 357.
21 Mollin J, Kasparek F & Lasovsky J, *Chem Zvesti*, 29 (1975) 39.
22 Dale L S & Jacob K, *J Am Chem Soc*, 76 (1954) 3297.
23 Albery W J & Davis M H, *J Chem Soc*, 68 (1972) 167.
24 Afanes'ev I B, *Superoxide Ion Chemistry & Biological Implications*, (CRC Press), 1989, p. 46.
25 Al-Ajlouni A, Bakac A & Espenson H J, *Inorg Chem*, 32 (1993) 5792.