Monatshefte für Chemie 136, 701–712 (2005)
DOI 10.1007/s00706-004-0273-8

Monatshefte für Chemie
Chemical Monthly
Printed in Austria

Preparation and Characterization of a Stable Semiquinone-Iron Complex

Wagner J. Barreto[1,*], **Sônia R. G. Barreto**[1], **Silvia Ponzoni**[4], **Yoshio Kawano**[2], **Eduardo Di Mauro**[3], **Hérica A. Magosso**[1], and **Waléria P. Silva**[1]

[1] Laboratory of Environmental Physical Chemistry, Chemistry Department, Londrina State University, 86051-990, Londrina, PR, Brazil
[2] Chemistry Department, University of São Paulo, CP 26077, 05513, São Paulo, SP, Brazil
[3] Department of Physics, CCE, Londrina State University, 86051, Londrina, Brazil
[4] Department of Physiology, CCB, Londrina State University, 86051-990, Londrina, PR, Brazil

Received June 24, 2004; accepted (revised) October 9, 2004
Published online March 16, 2005

Summary. Dopamine oxidation by iron oxide (Fe_2O_3) was studied in the presence and absence of sodium thiosulfate in aqueous medium around *pH* 7 by UV-Vis spectroscopy. The *pH* changes from 6 to 8 indicate that the dopamine oxidation process has occurred producing an anionic semiquinone radical which appears after *ca*. 100 hours presenting bands at 309 and 337 nm. It forms a stable compound with Fe(III) released by the iron oxide. The complex [*CTA*][Fe(SQ)$_2$(*CAT*)], where *SQ*=semiquinone, *CAT*=catecholate, and *CTA*=cetyltrimethylammonium cation, was isolated by precipitation with cetyltrimethylammonium bromide and was characterized through EPR, *Raman* and IR spectroscopies. The EPR spectrum presented two intense bands, one with $g = 2.003$ assigned to *o*-semiquinone and the other with $g = 4.274$ characteristic for high spin Fe(III) approaching an octahedral symmetry. The most intense *Raman* resonance band occurs at 1360 cm^{-1} assigned to $\nu(C_1–C_2)$ and at 1575 cm^{-1} to ν(C–C)ring of the *o*-semiquinone. The O_2 dissolved in solution is mainly responsible for the dopamine oxidation when sodium thiosulfate is present. A thermal decomposition mechanism based on the thermogravimetric curves (TG) was proposed. These results suggest that iron can participate in the degenerative process of the dopaminergic nigral neurons. Its role seems to be its coordination with the dopamine oxidation products as *o*-semiquinone and catecholate which could damage neurons giving rise to parkinsonism.

Keywords. Spectroscopy; EPR spectroscopy; *Raman* spectroscopy; Calorimetry.

* Corresponding author. E-mail: barreto@uel.br

Introduction

Parkinson's disease, also known as paralysis agitans, is a neurodegenerative disease characterized pathologically by progressive loss of catecholaminergic neurons in the substantia nigra. The degeneration of dopaminergic neurons and the resulting dopamine deficiency in the striatum are the neurological basis of the movement disorders characterizing *Parkinson*'s disease. The severity of the clinical picture observed in *Parkinson*'s disease presents a positive correlation with dopamine depletion levels. The cause of nigral neuronal death is still unknown. There are suggestions that degeneration of these neurons may be due to an active toxic process involving reactive oxygen species, the ''oxidative stress'' hypothesis. Dopamine within nigral neurons undergoes spontaneous autooxidation to neuromelanin, this process generates free radicals and neuromelanin itself may contain potentially toxic quinones and hydroxyquinones [1]. The study of dopamine oxidative mechanisms is important for the etiology of *Parkinson*'s disease.

The dopamine auto-oxidation reaction was studied widely and the proposed mechanism (Fig. 1) presents as final product an insoluble compound similar to melanin [2–4]. The action of transition metals to accelerate the oxidation process has been studied extensively. *Barreto et al.* [5] studied the aminochromes obtained from the dopamine, adrenaline, noradrenaline, and *L*-dopa oxidation with Mn(III) by resonance *Raman* spectroscopy. In another study a mechanism was proposed for dopamine, adrenaline, noradrenaline, and *L*-dopa oxidation using manganese oxide (MnO_2) in the presence of sodium thiosulfate [6].

An elevated amount of iron has been observed in cerebral tissues from patients with several types of neurodegenerative diseases such as *Parkinson*'s and *Huntington*'s diseases [7]. In the human brain iron is present in larger concentrations mainly in the substantia nigra, globus pallidus, red nucleus, and striatum [8]. 6-Hydroxydopamine (6-HO*DA*), a by-product of dopamine auto-oxidation, is used as toxic agent to nigral dopaminergic neurons in rat models of parkinsonism. The iron content is increased in the striatum of 6-HO*DA*-lesioned rats [9]. The increase in the iron content caused by 6-hydroxydopamine suggests that its accumulation could not be the primary cause of the neurodegenerative process observed in *Parkinson*'s disease [10]. The potential pathogenicity of iron in *P*D is related to

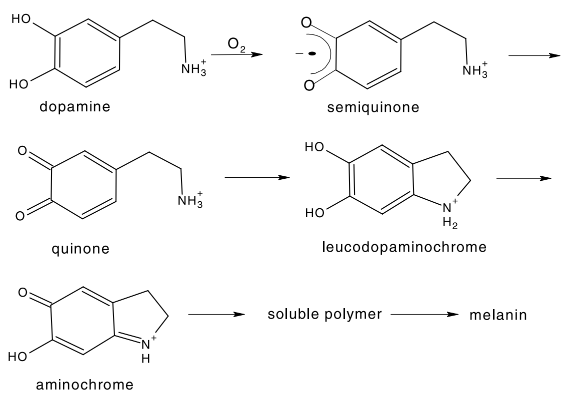

Fig. 1. Schematic representation of the auto-oxidation of dopamine as reported in the literature

its ability to generate free radicals and its selective binding to neuromelanin, producing Fe(III)-melanin complexes that may in turn induce oxidative stress [11]. A specific loss of melanized dopamine neurons from the substantia nigra is observed in *Parkinson*'s disease, followed by an increased concentration of ferric iron and copper in the tissue [12]. *Linert* and *Jameson* proposed a mechanism for the role of iron and 6-HO*DA* to explain the development of *Parkinson*'s disease. Iron(II) interacts via *Fenton*'s reaction producing OH-radicals that oxidize dopamine to 6-HO*DA*. The 6-HO*DA* reduces and releases iron(II) from the protein ferritin where the iron is stored in the form of a micro-crystalline structure built from FeO(OH) units [13].

The coordination chemistry of catechol and catecholamines with metals, particulary iron, has been studied extensively as structural and functional models for biochemical purposes [14–19].

The study of the dopamine oxidation process with iron oxide can become a model to understand the oxidative process that occurs naturally in healthy neurons that have become injured.

The preparation of a stable Fe(III) complex with radical ligands derived from dopamine is important in the study of the stress oxidative hypothesis as trigger to dopaminergic cell death. This paper reports the formation of [*CTA*][Fe(*SQ*)$_2$(*CAT*)] (*SQ*=semiquinone, *CAT*=catecholate, and *CTA*=cetyltrimethylammonium cation) in aqueous solution at *pH* 6–7, as well as its isolation and characterization in the solid state.

Results and Discussions

Study of Dopamine Oxidation in Solution

Figure 2A presents the UV-Vis spectra following the reaction of dopamine and iron oxide from 0 to 184 hours through the band at *ca.* 280 nm, an L_a–L_b coincident transition [5], characteristic of the internal transitions of dopamine. Appreciable

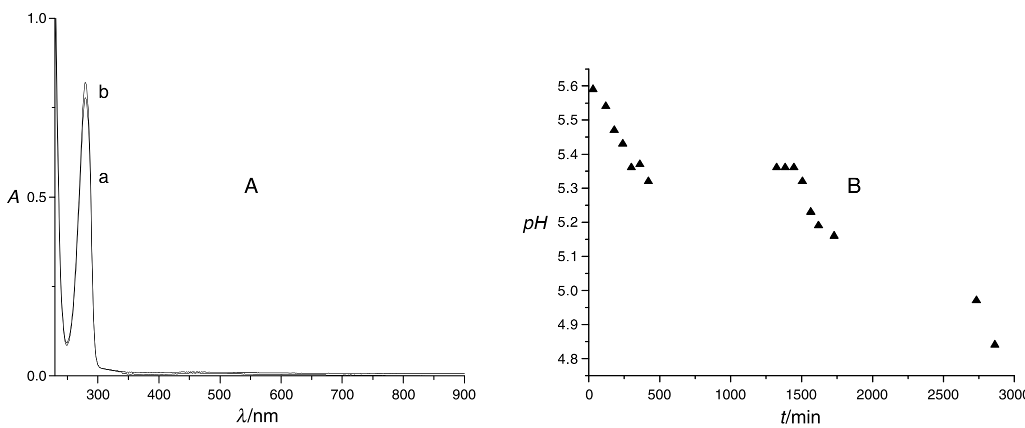

Fig. 2. (A) UV-Vis spectra of the aqueous reagent solution containing dopamine (1.15 mmol · dm^{-3}) and iron(III) oxide (0.315 mmol · dm^{-3}); (a) 0 and (b) 184 hours; (B) *pH* variation with reaction time

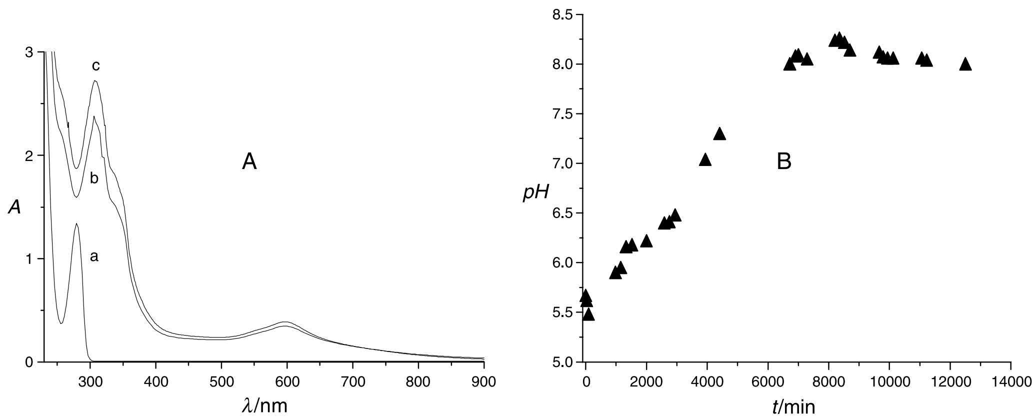

Fig. 3. (A) UV-Vis spectra of the aqueous reagent solution containing dopamine ($1.15\,mmol \cdot dm^{-3}$), iron(III) oxide ($0.315\,mmol \cdot dm^{-3}$), and sodium thiosulfate ($0.5\,mmol \cdot dm^{-3}$); (a) 0, (b) 71, and (c) 95 hours; (B) *pH* variation with reaction time

variation has not been observed in the intensity of the band while the *pH* decreases strongly from *pH* 6 to 4.8 (Fig. 2B).

When dopamine and iron oxide react in an aqueous medium in the presence of sodium thiosulfate (Fig. 3A) the band at 280 nm decreases, and an intense band at *ca*. 309 nm, a shoulder at 337 nm, and a weak band at 598 nm emerge. The *pH* variation in function of the time (Fig. 3B) shows an increase in *pH* from 5 to 8.5 being stabilized at *pH* 8.

Therefore when sodium thiosulfate is present in solution it catalyses the dopamine oxidation. The mechanism is complex with the *pH* increasing profile very similar to that of the catecholamines' reaction (dopamine, adrenaline, noradrenaline, *L*-dopa) with MnO_2 [6]. However, the reaction time for complete dopamine oxidation using iron oxide is much longer compared to manganese oxide (*ca*. 10 hours).

To demonstrate the thiosulfate importance to the dopamine oxidation process, a solution in which iron is absent was prepared. The UV-Vis spectra and *pH* variation have the same feature that is observed when iron oxide is present. After 209 hours the band at 280 nm disappeared, a band emerged at 307 nm and a shoulder at 332 nm, and the *pH* increased from 6 to 8.5.

The influence of O_2 for the dopamine oxidation in the presence of $S_2O_3^{2-}$ was investigated during 1274 hours. In absence of O_2 the intensity of the band at 280 nm did not vary nor did the *pH* (*pH* 4.4 for $t = 0\,h$ and *pH* 4.3 for $t = 1274\,h$) indicating that the dopamine had not been oxidized.

Therefore O_2 is the most important oxidizing agent in the dopamine, iron oxide, and sodium thiosulfate containing system. Atmospheric O_2 is dissolved in the aqueous solution during the long reaction time under continuous stirring. Normally the catecholamines undergo autooxidation using O_2 (Fig. 1) in the first reaction steps giving semiquinones and dopamine-quinones which further react to dopaminochrome and H_2O_2, producing melanin as the final product. The Fe(III) present in the solution has no influence on the rate of autooxidation [10]. In the reaction in the presence of sodium thiosulfate, however, the intermediate *o*-semiquinone is stabilized which, after the dopamine oxidation, prevents further oxidation steps.

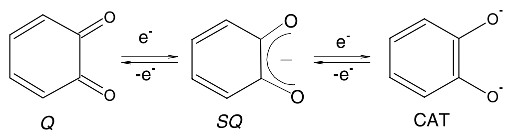

Fig. 4. The inter-conversion oxidation state scheme among catecholate (*CAT*), semiquinone (*SQ*), and quinone (*Q*) forms

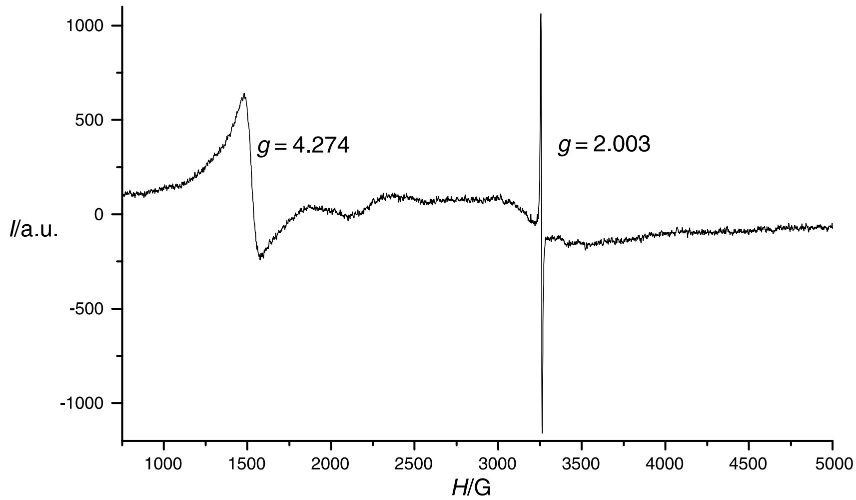

Fig. 5. EPR spectra of the solid complex [*CTA*][Fe(SQ)$_2$(*CAT*)] isolated from the solution containing dopamine (1.15 mmol · dm^{-3}), iron(III) oxide (0.315 mmol · dm^{-3}), and sodium thiosulfate (0.5 mmol · dm^{-3}); EPR spectrum was obtained at the X-band (9.5 GHz) microwave frequency and with a magnetic field modulation of 100 kHz at room temperature

Characterization of the Iron Complex in the Solid State

The compound formed in solution containing dopamine, Fe_2O_3, and sodium thiosulfate was isolated from the solution using cetyltrimethylammonium bromide as precipitant. The oxidation state of the ligand is difficult to determine because the dioxolenes present a low inter-conversion energy barrier among the catecholate, semiquinone, and quinone forms (Fig. 4).

The EPR spectrum (Fig. 5) shows the presence of two intense signals, one with $g = 2.003$, of a dopamine derived radical, *i.e.*, an *o*-semiquinone (*SQ*) and another with $g = 4.274$, characteristic of the iron(III) high spin in near octahedral geometry. The literature relates that iron(III)-*SQ* complexes show strong antiferromagnetic spin–spin coupling of Fe–*SQ* or *SQ*–*SQ*, therefore they should be EPR silent [20, 21]. We believe that the mixed nature of the complex, with SQ^- and CAT^{2-} ligands, resulted in weaker spin–spin coupling and so the iron EPR signal could appear. The band at 309 nm is normally assigned to the semiquinone radical anion for dopamine and the band at 337 nm could be assigned to the catechol ligand (*CAT*), and the complex could be represented as [*CTA*][Fe(SQ)$_2$ (*CAT*)] (Fig. 6).

The *Raman* spectrum (Fig. 7A) reveals intense bands at 1360 cm^{-1} and 1575 cm^{-1}. These frequency values are very close to those obtained for the

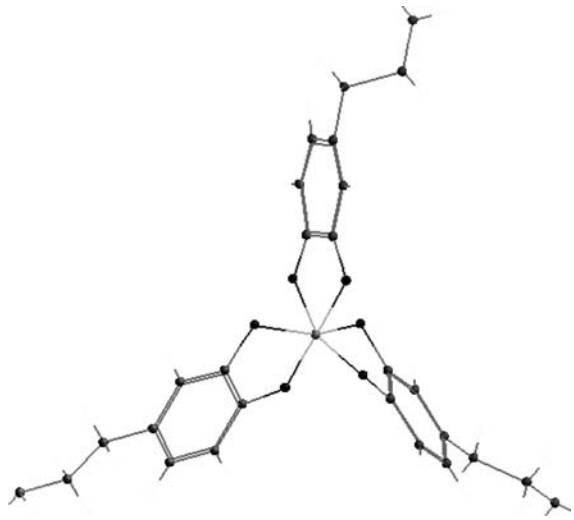

Fig. 6. A schematic structure of the complex [*CTA*][Fe$(SQ)_2$(*CAT*)]

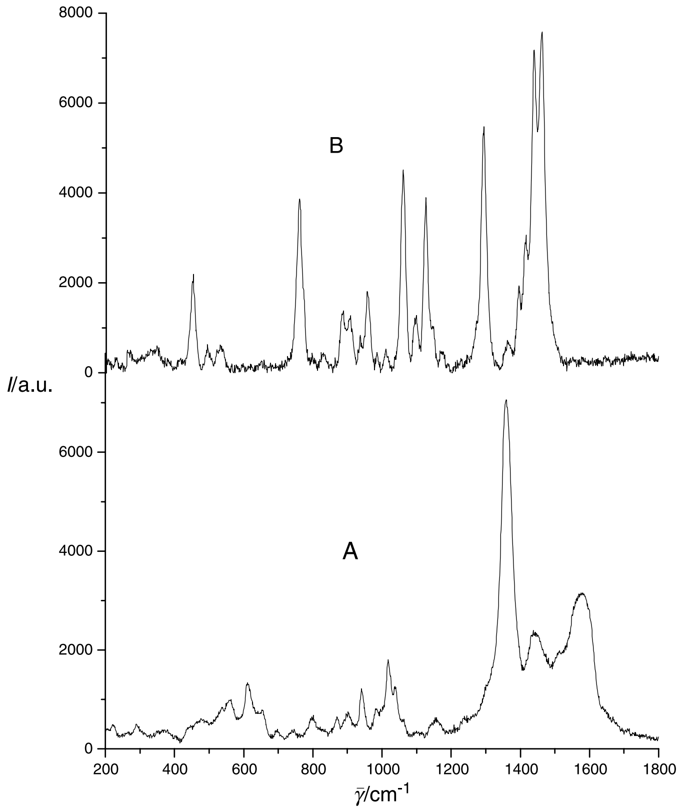

Fig. 7. *Raman* spectra of the complex [*CTA*][Fe$(SQ)_2$(*CAT*)] in solid state (A) and the solid cetyltrimethylammonium bromide salt (B); the *Raman* spectra were obtained with 488 nm radiation, 150 mW laser power, and spectral resolution of 7 cm^{-1}

Table 1. Observed *Raman* and IR wavenumbers (cm^{-1}) and a tentative assignment based on a PM3 semi-empirical calculation for the complex [*CTA*][Fe(SQ)$_2$(*CAT*)]

Assignment	[*CTA*][Fe(SQ)$_2$(*CAT*)]		*CTAB*		Calculate/cm^{-1}
	Raman/cm^{-1}	IR/cm^{-1}	*Raman*/cm^{-1}	IR/cm^{-1}	
CTAB		3020 w		3024 w	
CTAB		2920 vs		2926 vs	
CTAB		2851 vs		2858 vs	
		1634 m		1638 m	
ν_{ring}	1576 s				1594
				1494 s	
CTAB		1487 m		1481 s	1514
CTAB		1465 m	1461 vs	1471 s	
CTAB	1441 m	1441 vw	1439 vs	1441 vw	
			1414 sh m	1416 vw	
$\nu_{C_1C_2}$, ν_{CO}	1360 vs	1385 m			1400
			1294 s		
ν_{CO}		1224 s			1270
β(CH)	1159 vw				
ν_{CO}		1171 sh m			
β(CH)		1110 w			
ν_{ring}	1039 sh w				
ν_{ring}	1020 m	1026 s			
			1126 s		
			1097 m		
			1062 s		
ν_{ring}	984 w				1007
			985 vw		
CTAB		963 w	958 m	962 m	
ν_{ring}	942 m				1004
CTAB		938 w	935 vw	938 w	
ν_{ring}	901 w				923
CTAB		911 w	905 m	913 m	
			886 m		
ν_{ring}	870 w				
ν_{ring}	800 w				
ν_{ring}	744 w				
ν_{ring}	697 w				
			761 s		
				773 m	
				730 m	
CTAB		723 w		722 m	
				670 m	
ν_{ring}, ν_{Mring}	655 sh w	650 m		652 m	719
ν_{ring}, ν_{Mring}	612 m	625 sh m			636
	560 m				
	479 w				
	370 w				

(*continued*)

Table 1 (*continued*)

Assignment	[*CTA*][Fe(SQ)$_2$(*CAT*)]		*CTAB*		Calculate/cm^{-1}
	Raman/cm^{-1}	IR/cm^{-1}	*Raman*/cm^{-1}	IR/cm^{-1}	
	292 w				
		538 w			
			532 w		
			493 w		
			451 s		

s = strong, vs = very strong, w = weak, vw = very weak, sh = shoulder, m = medium

manganese complexes with dopasemiquinone and *L*-dopasemiquinone in aqueous solution being found by resonance *Raman* spectroscopy at 1373 and 1377 cm^{-1} [6] and in solid state at 1360 and 1356 cm^{-1} [22].

Table 1 presents the IR and *Raman* wavenumbers observed and calculated with a PM3 semi-empirical method. A general criterion present in the literature was used to assign the bands of these dioxolene complexes. It is well known that the frequencies for the C–O bond are observed between 1630–1640 cm^{-1} for *M-Q*, 1400–1500 cm^{-1} for *M-SQ*, and 1250–1275 cm^{-1} for *M-CAT* complexes [23]. Therefore the most intense *Raman* band at *ca*. 1360 cm^{-1} could be assigned to a C–O stretching with major C_1–C_2 character (C_1 and C_2 are the carbons bonded to oxygen) remaining close to that assigned for the *M-SQ* (1400 cm^{-1}). It is difficult to specify the C–O contribution to this mode, but it is reasonable to use this mode and the absence of the intense *Raman* band at *ca.* 1480 cm^{-1} to characterize the ligands as *o*-semiquinone radical anions. We can tentatively assign the resonance *Raman* band observed at *ca*. 1575 cm^{-1} to a ring stretching, and the broad band at the 400–750 cm^{-1} region can be assigned to deformations associated with the five-member ring chelate including the iron ion, oxygen, and C_1–C_2 bonds. The absence of a strong *Raman* resonance band at *ca*. 1440 cm^{-1} assigned as $\nu(C{=}N^+)$ for all chrome substances, such as aminochrome, indicates that cyclization of the aminoethyl side chain did not occur.

The IR spectrum (Fig. 8A) showed that most bands of the spectrum could be assigned to the cetyltrimethylammonium cation. However, there is a region between 980 and 1380 cm^{-1} where the cation did not absorb. The band at 1226 cm^{-1} is close to the expected frequencies for the catecholate complex with transition metals related in the literature to 1250–1275 cm^{-1} for ν(C–O) and 1480 cm^{-1} for ν(ring) [23].

The TG curve was obtained for the complex (Fig. 9) and a thermal decomposition was proposed.

$$\begin{array}{lll}
2\{[CTA][Fe(SQ)_2(CAT)]\cdot H_2O\} & \rightarrow & 2\{[CTA][Fe(SQ)_2(CAT)]\} + 2H_2O \\
2\{[CTA][Fe(SQ)_2(CAT)]\} & \rightarrow & 2\{[CTA][Fe(O)(SQ)(CAT)]\} \\
 & & +2SQ(\text{less 2 oxygen}) \\
2\{[CTA][Fe(O)(SQ)(CAT)]\} & \rightarrow & 2\{[CTA][Fe(O)_3(SQ)]\} + 2CAT \\
 & & (\text{less 4 oxygen})
\end{array}$$

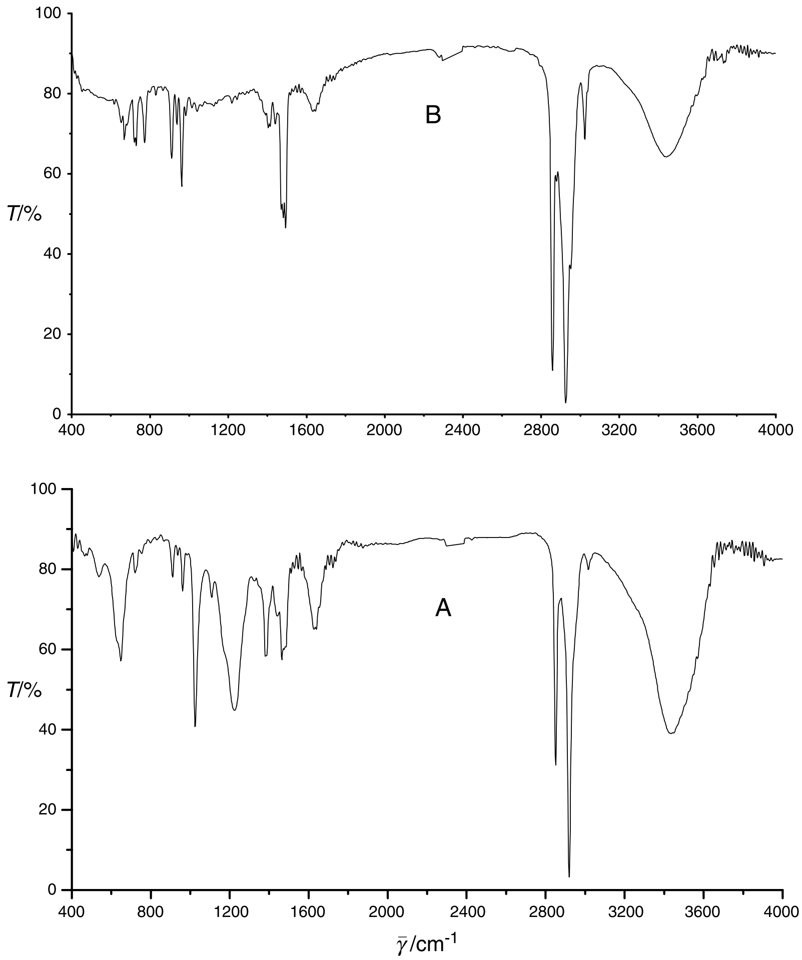

Fig. 8. Infrared spectrum of the solid complex [*CTA*][Fe(*SQ*)$_2$(*CAT*)] in KBr pellets (1:100) (A) and the solid cetyltrimethylammonium bromide salt (B); the spectral resolution was 4 cm^{-1} and 80 spectra were accumulated

$$\begin{array}{lll} 2\{[CTA][\mathrm{Fe(O)_3}(SQ)]\} & \rightarrow & \mathrm{Fe_2O_8} + 2[CTA] + 2\mathrm{SQ(less\ 2\ oxygen)} \\ \mathrm{Fe_2O_8} & \rightarrow & \mathrm{Fe_2O_3} + 5/2\mathrm{O_2} \\ \mathrm{Fe_2O_3} & \rightarrow & \mathrm{residue} \end{array}$$

The mass losses for each step according to the mechanism above were, calculated and experimental, respectively: step 1 (2.0, 1.8%), step 2 (17.6, 22.2%), step 3 (13.4, 12.7%), step 4 (47.5, 41.6%), step 5 (4.9, 5.6%), and step 6 (14.6, 16.3%). There is uncertainty about the values obtained because of experimental errors in the choice of the temperature intervals for each mass loss step. The mechanism implies that there is a difference in the release of *SQ* and *CAT*. The SQ ligand released one oxygen atom from each benzene but one remained bonded to the iron atom while for the CAT ligand all the oxygen remained bonded to the iron during the thermal decomposition.

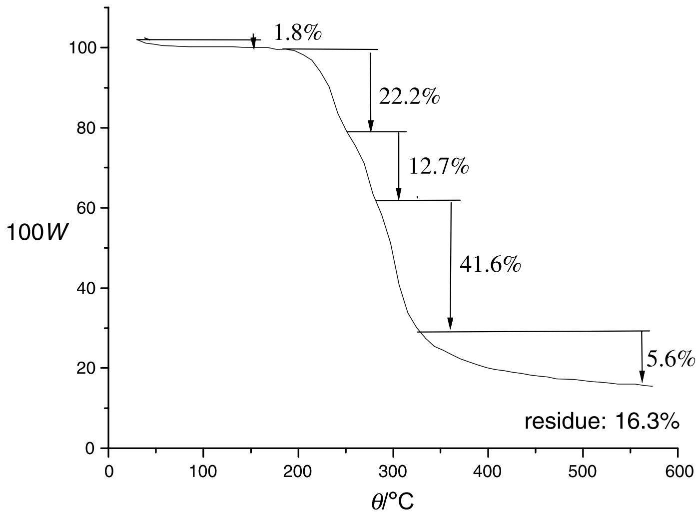

Fig. 9. TG curves for the solid complex [*CTA*][Fe(*SQ*)$_2$(*CAT*)]; the mass losses (TG) were obtained with N_2 atmosphere and a heating rate of 10°C/min

Conclusion

The present study revealed that iron(III) oxide did not oxidize dopamine in aqueous media (*pH* 6–7) in the presence of sodium thiosulfate. The oxidative action of the oxide on the dopamine was quite reduced, generating however Fe(III) in solution that coordinates with the *o*-semiquinone and catecholate products of the dopamine oxidation. The Fe(III) appeared in solution as a result of the parallel reaction of iron oxide reduction due to the long time reaction. The main agent in dopamine oxidation is the dissolved oxygen present in aqueous solution. These two products are long-standing in solution due to the presence of sodium thiosulfate that stabilized the complex preventing the polymerisation that generates melanin. The ligand radical in this complex presents stability above expectations and remained in the complex isolated in solid state. Contrary to the [*CAT*][Mn(*SQ*)$_3$] complex, *SQ*=dopasemiquinone or *L*-dopasemiquinone, that in solution presented an intense charge transfer band around 600 nm, the Fe(III) complex exhibits a less intense band.

The EPR, *Raman*, and IR spectra of the solid complex showed that the *o*-semiquinone is present in the compound and the EPR spectrum also confirms the presence of Fe(III).

The results suggest that iron can participate in the degenerative process of the dopaminergic nigral neurons due to the coordination with the dopamine oxidation products, like *o*-semiquinone and catecholate, damaging healthy neurons and giving rise to parkinsonism.

Experimental

Reagents

Dopamine ($C_8H_{11}O_2N$, Aldrich Chem. Co. 98%), iron oxide (Fe_2O_3, Aldrich Chemical Co. 99,98%), cetyltrimethylammonium bromide ($C_{19}H_{42}N$, Fluka Chemika 98%), sodium thiosulfate ($Na_2S_2O_3$, Aldrich Chemical 99%), chloroform (Merck 99%), standard iron solution (Merck), HNO_3 (Merck 65%).

Solution Preparations

All solutions were prepared with ultra-pure water at a temperature of 25 ± 2°C.

Dopamine and iron oxide solution. Dopamine (0.1086 g, $1.15\times10^{-3}\ mol \cdot dm^{-3}$) was added to water (500 cm^3). An UV-Vis absorption spectrum (time 0) between 200 and 900 nm was obtained. Iron oxide (0.0251 g, $3.15.10^{-4}\ mol \cdot dm^{-3}$) was added and the UV-Vis spectrum and *pH* at several reaction times was determined.

CTAB solution. Cetyltrimethylammonium bromide (0.2010 g, $2.74.10^{-2}\ mol \cdot dm^{-3}$) was added to 20 cm^3 of water, dissolved by means of ultrasound and *ca.* 10 cm^3 of this solution were used for precipitation.

Dopamine, sodium thiosulfate and iron oxide solution. For this preparation the same procedure as for dopamine with iron oxide was used, but sodium thiosulfate (0.3010 g, $2.50.10^{-3}\ mol \cdot dm^{-3}$) was added to the solution. The order used to mix the reagents was: sodium thiosulfate, dopamine, and iron oxide. UV-Vis spectra were taken at intervals and *pH*-time dependencies were measured.

Preparation of the solid iron complex. A solution with dopamine, sodium thiosulfate, and iron oxide was kept monitoring the band at 280 nm, characteristic of dopamine. After *ca.* 193 hours, with the complete disappearance of the band at 280 nm, a band emerged at 309 nm indicating the end of the reaction. The solution was centrifuged for 20 min at 8000 rpm for elimination of the iron oxide, and the cetyltrimethylammonium bromide solution (10 cm^3) was added slowly and under agitation. The solution was transferred to a separation funnel and chloroform (50 cm^3) was added. It was agitated for 5 min and the organic phase was extracted. The organic phase was transferred to a beaker and the chloroform was allowed to evaporate at room temperature, and finally the sample was dried in a desiccator. It was not possible to obtain the complex in crystalline form suitable for crystallographic studies. The complex presents the formula $[CTA][Fe(SQ)_2(CAT)]$ in which *SQ*=*o*-semiquinone, *CAT*=catecholate, and *CTA*=cetyltrimethylammonium cation. Elemental analyses (C, H, N, Fe) were conducted using the Elemental Analyser Perkin Elmer; their results were found to be in good agreement with the calculated values.

Dopamine and sodium thiosulfate without O_2. The solution was prepared containing dopamine (1.06 $mmol \cdot dm^{-3}$) and sodium thiosulfate (2.42 $mmol \cdot dm^{-3}$), but the flask was maintained stoppered and covered with a *PVC* film to suppress oxygen entrance. The UV-Vis spectra and *pH* were obtained before covering and after the flask was opened (1274 hours of reaction).

Physical Measures

The solutions were maintained under agitation at 25°C using magnetic stirring (Microquímica, MQAMA 301). The *pH* was measured using a *pH*-meter (Hanna Instruments, HI 9321). The chemical reaction was followed spectrophotometrically (Milton-Roy Genesys 2) in the UV-Vis region using a quartz cuvette with a 1 cm optical path under a controllable temperature at 25°C.

The infrared spectra (SHIMADZU FT-IR spectrophotometer) were obtained at room temperature from 400 to 4000 cm^{-1}, KBr pellets (1:100), resolution of 4 cm^{-1}, and accumulation of 80 spectra.

EPR experiments were performed at the X-band (9.5 GHz) microwave frequency and with a magnetic field modulation of 100 kHz using a VARIAN E-109 apparatus at room temperature. The microwave frequency was accurately read with a Hewlett Packard frequency counter, model HP 5352B. The data were acquired with a PC microcomputer using software for data acquisition developed at the Institute of Physics of the University of São Paulo at São Carlos, Brazil.

The iron content in the complex was determined using an Atomic Absorption Spectrometer (SHIMADZU AA-6601F) with an iron lamp at 248.33 nm. A calibration curve was built with a standard iron solution from 0.1 to 6.0 mg · dm^{-3} in nitric acid 1%. Samples of the complex (5 mg) were digested with HNO_3 and diluted to 10 cm^3 with HNO_3 1% for measuring.

The *Raman* spectra were obtained with a Jobin-Yvon spectrometer, radiation at 488 nm, resolution of 7 cm^{-1}, and 150 mW laser power.

The mass losses (TG) were obtained in a T. A. Instruments TG 2950, High Resolution device, with N_2 atmosphere and a heating rate of 10°C/min.

Acknowledgements

The authors thank CNPq and Fundação Araucária for the financial support, *W. P. Silva* and *H. A. Magosso* for the PIBIC-CNPq program. The authors thank Prof. *O. Rangel de Nascimento* (Laboratory of Biophysical – USP, São Carlos) for EPR spectra and Prof. *M. L. A. Temperini* (Laboratory of Molecular Spectroscopy – IQ-USP, São Paulo) for *Raman* spectra.

References

[1] Gerlach M, Ben-Sachar D, Riederer P, Youdim MBHJ (1994) Neurochem **63**: 793
[2] Heacock RA (1965) Advan Heterocycl Chem **5**: 205
[3] Graham DG (1978) Mol Pharmacol **14**: 633
[4] Herlinger E, Jameson RF, Linert W (1995) J Chem Soc Perkin Trans 2, 259
[5] Barreto WJ, Ponzoni S, Sassi P (1999) Spectrochim Acta Part A**55**: 65
[6] Barreto WJ, Barreto SRG, Santos MA, Schimidt R, Paschoal FMM, Mangrich AS, deOliveira LFC (2001) J Inorg Biochem **84**: 89
[7] Good P, Olanow C, Perl D (1992b) Brain Res **593**: 343
[8] Riederer P, Dirr A, Goetz M, Jellinger K, Youdim MBH (1992) Ann Neurol **32**: 101
[9] Hall S, Rulledge JH, Schallert T (1992) J Neurol Sci **113**: 198
[10] Oestreicher E, Sengstock GJ, Riederer P, Olanow CW, Dunn AJ, Arendash GW (1994) Brain Res **660**: 8
[11] Kienzl E, Puchinger L, Jellinger K, Linert W, Stachelberger H, Jameson RF (1995) J Neurol Sci **134** (Suppl): 69
[12] Zecca L, Shima T, Stroppolo A, Goj C, Battiston GA, Gerbasi R, Sarna T, Swartz HM (1996) Neuroscience **2**: 407
[13] Linert W, Jameson GNL (2000) J Inorg Biochem **79**: 319
[14] Yamahara R, Ogo S, Masuda H, Watanabe Y (2002) J Inorg Biochem **88**: 284
[15] Michaud-Soret I, Andersoson KK, Que L (1995) Biochemistry **34**: 5504
[16] Mialane PM, Anxolabéhère-Mallart E, Blondin G, Nivorojkine A, Guilhem J, Tchertanova L, Cesario M, Ravi N, Bominaar E, Girerd J-J, Munck E (1997) Inorg Chim Acta **263**: 367
[17] Karpishin TB, Gebhard MS, Solomon EI, Raymond KN (1991) J Am Chem Soc **113**: 2977
[18] Salama S, Stong JD, Neilands JB, Spiro TG (1978) Biochemistry **17, 18**: 3781
[19] Aouad F, Florence A, Zhang Y, Collins F, Henry C, Ward RJ, Crichton RR (2002) Inorg Chim Acta **339**: 470
[20] Attia AS, Conklin BJ, Lange CW, Pierpont CG (1996) Inorg Chem **35**(4): 1033
[21] Pierpont CG (2001) Coord Chem Rev **219**: 415
[22] Barreto WJ, Barreto SRG, Kawano Y, deOliveira LFC, DiMauro E, Paschoal FMM (2003) Monatsh Chem **134**: 1545
[23] Vlcek A Jr (1994) Comments Inorg Chem **16**: 207