Formation of Tris-chelated Vanadium(IV) Complexes by Interaction of Oxovanadium(IV) with Catecholamines, 3-(3,4-Dihydroxyphenyl)alanine and Related Ligands in Aqueous Solution

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Complex formation between oxovanadium(IV) and a series of *o*-catecholic ligands was investigated in aqueous solution by EPR and electronic absorption spectroscopy and pH-potentiometry. The reaction scheme involves, besides the normal mono- and bis-catecholato oxovanadium(IV) complexes, also species where the bare vanadium(IV) ion, lacking the oxo ligand, is chelated by three catecholate ligands. A comparative examination of the systems showed that the extent to which the ligands are able to form tris complexes with V^{IV}, by displacing the oxo group from VO^{2+}, may be reasonably interpreted in terms of the electrostatic repulsion between the bis complex and the attacking third ligand molecule and also the base strength of the phenolate groups of the ligands.

The chemistry of vanadium in biological systems is characterised by the ability of the element to enter living organisms and the easy interconversion between its +4 and +5 oxidation states. Vanadate(V) can enter the cell, much more effectively than oxovanadium(IV), by using specific or non-specific (*e.g.* phosphate) transport systems. Once inside, it inhibits enzymes which catalyse phosphoryl-transfer reactions, *e.g.* Na^+-, K^+-ATPases.[1] Small biomolecules are likely to reverse these toxic effects by reducing vanadium(V) to oxovanadium(IV) and/or complexing the reduced ions. This class of biomolecules includes, among others, catecholic compounds which are well known as reducing and complexing agents of vanadium in the +5 and +4 oxidation states, respectively.

One of the most typical features of the *o*-catechol–vanadium system is the ability of the ligand to stabilise bare vanadium(IV) species by displacing the co-ordinated oxo group and forming, even in aqueous solution, tris-chelated complexes exhibiting a six-co-ordinate geometry with severe trigonal-prismatic distortion.[2–5] The most effective mechanism for such a process involves the reaction of a ligand molecule, having the *o*-diphenolic site undissociated, with the bis-chelated catecholato complex of oxovanadium(IV) and the formation of a water molecule. Accordingly, the tris(catecholato) complex is hindered by the presence of other oxovanadium(IV) binding sites in the ligand, like carboxylate group(s) in 2,3-dihydroxybenzoic acid,[6] or 2,3-dihydroxyterephthalic acid and 2,3,4-trihydroxybenzoic acid.[7] Nevertheless, the formation of such a type of complexes leading to the stabilisation of bare V^{IV} is a general reaction for catecholic ligands in natural systems. For instance, as shown by EPR spectra, similar complexes are easily formed, in the solid state, by tannic compounds which adopt three catecholic residues in metal binding.[8]

The reducing and complexing behaviour reported for some catecholamines[3,9,10] suggested that these ligands can act as primary antagonists of vanadium(V) in biological systems by contributing to maintain the enzymatic activity.[11,12] However, the data so far published about the interaction of oxovanadium(IV) with these ligands are rather incomplete. It was reported that vanadium(IV) complexes are formed upon reduction of V^V in the presence of norepinephrine [4-(2-amino-1-hydroxyethyl)benzene-1,2-diol] and epinephrine [4-(1-hydroxy-2-methylaminoethyl)benzene-1,2-diol], as demonstrated by the EPR signals generated during reduction.[9,13] On the other hand, the existence of tris-chelated complexes was postulated by Jameson and Kiss[3] in order to interpret the kinetics of the vanadium(IV)-catalysed oxidation of epinephrine. Since in the respective formation process no protons are consumed or liberated, these species seem to be undetectable by potentiometric titrations.

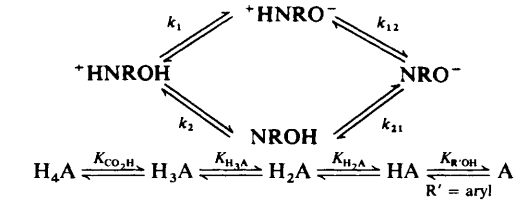
A study was therefore carried out with the aim of investigating in detail vanadium(IV)–catecholamine {dopamine [4-(2-aminoethyl)benzene-1,2-diol], D,L-epinephrine and D,L-norepinephrine} systems by pH-potentiometric investigation and following directly the formation of the tris(catecholato) complexes by EPR and absorption spectroscopy. The investigation was extended to the complexes of related ligands, namely Tiron (disodium 4,5-dihydroxybenzene-1,3-disulfonate) as well as the biochemical precursors of catecholamines, dopamine and L-dopa [3-(3,4-dihydroxyphenyl)alanine]. The comparative analysis could be of help in reaching conclusions about the factors leading to the stabilisation of tris-(catecholato) complexes of vanadium(IV).

Experimental

Materials.—The ligands were Aldrich or Sigma products of puriss. quality; $VOSO_4 \cdot 5H_2O$ was used as the metal salt.

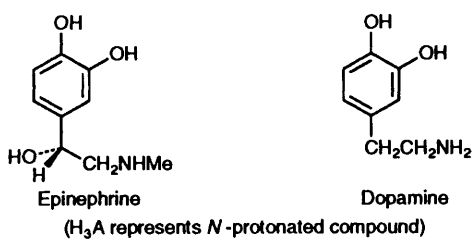
Potentiometric Measurements.—The stability constants of the vanadium(IV) complexes of the ligands were determined at 25 °C by pH-metric titration of 25 cm^3 samples. The concentration of the ligands was 4×10^{-3} or 2×10^{-3} mol dm^{-3} and the metal ion-to-ligand molar ratio was 0:2, 1:1, 1:2 or 1:4. Titrations were performed over the range pH 2.2–11.0 with a KOH solution of known concentration (*ca.* 0.2 mol dm^{-3}) under a purified argon atmosphere in order to avoid oxidation of the

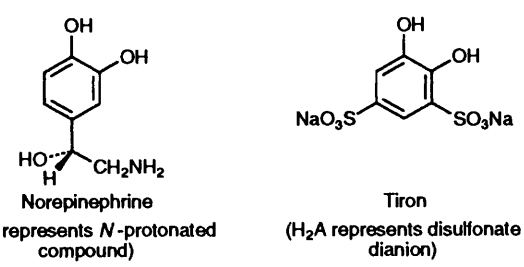
Table 1 Acid–base properties of catecholamines and other catechol derivatives at 25 °C and I = 0.2 mol dm^{-3} (KCl)

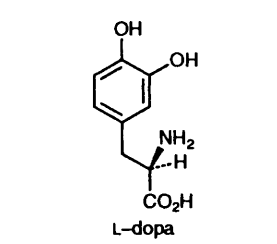


Ligand	pK_{ROH}	$pK_{R'OH}$	pK_{H_2A}	pK_{H_3A}	pk_1 [a]	pk_2 [a]	Ref.
o-Catechol	—	13.0	9.28	—	—	—	16
Tiron	—	12.2	7.47	—	—	—	5
Dopamine	—	13.1	10.41	8.89	8.90	9.98	17
Epinephrine	(14)	13.1	9.84	8.64	8.67	9.38	19
Norepinephrine	(14)	12.9	9.53	8.58	8.69	9.11	19
L-dopa [b]	—	13.4	9.83	8.80	8.98	9.19	18

[a] pk_1 and pk_2 are microconstants, characteristic of deprotonation of the phenolic OH and side-chain NH_3^+ groups, respectively. [b] pK_{CO_2H} = 2.22.







ligands. Other conditions were as described earlier.[5] The pH was measured with a Radiometer pHM 84 instrument, with a G2040B glass and K4040 calomel electrodes. The electrode system was calibrated for hydrogen-ion concentration according to Irving *et al.*[14] A pK_w value of 13.76 was determined and used for the titrations at 25 °C and I = 0.20 mol dm^{-3}. Concentration stability constants $\beta_{pqr} = [M_pA_qH_r]/[M]^p[A]^q[H]^r$ were calculated by the PSEQUAD computer program.[15]

Spectroscopic Measurements.—Isotropic and anisotropic X-band EPR spectra (9.15 GHz) were recorded at 298 or 140 K, respectively, on aqueous solutions using a Varian E-9 spectrometer. As usual, the samples for low-temperature measurements were added with a few drops of dimethyl sulfoxide (dmso) to ensure good glass formation in frozen solutions. The dmso did not appear to affect the spectra, unlike, *e.g.*, ethylene glycol, which is known to interact with VO^{2+} over the high pH range. Absorption spectra were recorded with a Beckman Acta MIV spectrophotometer. All manipulations and titrations were made under an atmosphere of nitrogen.

Results and Discussion

The acid–base properties of the ligands were studied earlier.[5,16–19] Here only a list of the dissociable groups with their pK values is given in Table 1 in order to assist the interpretation of the metal-binding properties of the ligands. Estimated values are given in parentheses for those pK values which could not be determined. For catecholamines and L-dopa the microconstants (pk) of the overlapping deprotonation processes for the side-chain ammonium group and the first phenolic hydroxyl group are also included in Table 1.

The equilibria corresponding to the formation of the hydroxo complexes of VO^{2+} were taken into account in the calculation of the stability constants of the complexes. The following species were assumed: $[VO(OH)]^+$ (log $\beta_{1-1} = -5.94$), $[\{VO(OH)\}_2]^{2+}$ (log $\beta_{2-2} = -6.95$), with stability data calculated from the data published by Henry *et al.*[20] using the Davis equation to take into account the different ionic strengths, and $[VO(OH)_3]^-$ (log $\beta_{1-3} = -18.0$), taken from ref. 21. Owing to the high stability of the vanadium(IV) catecholate interaction, hydroxo-complex formation, however, remained negligible in the whole pH range studied.

The stability constants of the complexes formed in the vanadium(IV)–ligand systems are listed in Table 2, isotropic ESR parameters and electronic absorption maxima are given in Table 3.

VO^{IV}–*Tiron.*—The compound Tiron behaves as an *o*-diphenolic ligand, as is clearly demonstrated by both the spectral and pH-metric measurements. From the sequence of EPR spectra recorded at room temperature on aqueous solutions of VO^{2+} and ligand, as a function of pH and ligand-to-metal molar ratio, the mononuclear species taking part in the complex-formation equilibria can be identified and are in fair accord with earlier proposals.[2,22] The results are also in good agreement with potentiometric and spectrophotometric data published previously.[5] Namely, at the ligand-to-metal molar ratio of 2:1, the $[VO(H_2O)_5]^{2+}$, identified by the measured values of the isotropic splitting factor g_0 and hyperfine coupling constant A_0 (^{51}V, $I = \frac{7}{2}$), is detected below pH 2.5. At pH 2.5 this is still a major species, but it coexists with a complexed species which becomes predominant around pH 4 (see Fig. 1). By comparison with the analogous catechol–vanadium(IV) system,[4,6] the species is assigned as the 1:1 complex $[VOA]^{2-}$, in which the ligand acts as a bidentate chelated donor through the *o*-diphenolate site. Around pH 5 a second complex is detected and

Table 2 Stability constants (log β) for vanadium(IV) complexes of catecholamines and L-dopa at 25 °C and I = 0.2 mol dm^{-3} (KCl)

	Dopamine	D,L-Epinephrine	D,L-Norepinephrine	L-dopa
$[VOAH_2]$	—	—	—	30.05(4)
[VO(HA)]	27.10(1)	26.64(1)	26.09(1)	26.88(1)
[VOA]	21.34(8)	—	—	21.34(2)
$[VO(HA)_2]$	52.65(1)	51.59(2)	50.35(2)	51.67(2)
$[VOA_2H]$	42.42(2)	41.92(3)	41.09(4)	42.18(4)
$[VOA_2]$	31.75(1)	31.82(2)	31.40(3)	31.79(7)
$[VOA_3H_5]$[a]	87.26(8)	85.42(7)	83.60(13)	86.24(7)
$\log K_{VO(HA)_2}$	25.45	24.95	24.26	24.79
$pK_{VO(HA)_2}$	10.23	9.67	9.26	9.49
pK_{VOA_2H}	10.67	10.10	9.69	10.39
log K^{*}[b]	2.2	2.3	2.2	2.5

[a] $[VOA_3H_5] = [V(HA)_3]\cdot H_2O$. [b] K^{*} refers to the reaction $[VO(HA)_2] + H_2(HA) \rightleftharpoons [V(AH)_3]\cdot H_2O$; $\log K^{*} = \log \beta_{VOA_3H_5} - \log \beta_{VO(HA)_2} - \log \beta_{H_3A}$.

Table 3 Isotropic EPR parameters and electronic absorption maxima of vanadium(IV) complexes of catecholamines and other catechol derivatives

Ligand	Complex	g_0	$10^4 A_0$/cm^{-1}	Absorption maxima[a]/nm	Ref.
o-Catechol	[VOA]	1.968	96	550 (43), 710 (43)	6
	$[VOA_2]^{2-}$	1.975	82	550 (50), 662 (77)	6
	$[VA_3]^{2-}$	1.955	76	552 (9200), 650 (8200)	2
Tiron	$[VOA]^{2-}$	1.973	95	525 (14), 750 (20)	*b*
	$[VOA_2]^{6-}$	1.977	81	530 (35), 650 (61)	*b*
	$[VA_3]^{8-}$	1.940	85	560 (5400), 650 (4800)	*b*
Dopamine	$[VO(HA)]^{+}$, [VOA]	1.975	96	*c*	*b*
	$[VO(HA)_2]$, $[VOA_2H]^{-}$, $[VOA_2]^{2-}$	1.976	82	*c*	*b*
	$[V(HA)_3]^{+}$	1.931	84	595 (6300), 660 (5800)	*b*
Epinephrine	$[VO(HA)]^{+}$	1.972	95	*c*	*b*
	$[VO(HA)_2]$, $[VOA_2H]^{-}$, $[VOA_2]^{2-}$	1.976	82	*c*	*b*
	$[V(HA)_3]^{+}$	1.934	84	590 (7200), 660 (6400)	*b*
Norepinephrine	$[VO(HA)]^{+}$	1.968	95	*c*	*b*
	$[VO(HA)_2]$, $[VOA_2H]^{-}$, $[VOA_2]^{2-}$	1.974	82	*c*	*b*
	$[V(HA)_3]^{+}$	1.935	85	580 (7500), 660 (6900)	*b*
L-dopa	[VO(HA)], $[VOA]^{-}$	1.972	95	*c*	*b*
	$[VO(HA)_2]^{2-}$, $[VOA_2H]^{3-}$, $[VOA_2]^{4-}$	1.976	81	*c*	*b*
	$[V(HA)_3]^{2-}$	*d*	*d*	600 (5700), 660 (5300)[d]	*b*

[a] Molar absorption coefficients (dm^3 mol^{-1} cm^{-1}), referred to total metal concentration for solutions where the complex species is predominantly formed, are given in parentheses. [b] This work. [c] Not measurable due to the presence of small amounts of tris complex even in solutions at low ligand-to-metal molar ratio. [d] Not measurable with the same precision as in the other systems due to the incomplete formation of the complex as an effect of the low solubility of the ligand.

becomes the only oxovanadium(IV) species from pH 6.4 up to 12. This species is assigned as the bis-chelated catecholato complex $[VOA_2]^{6-}$. The lack of a significant decrease in EPR intensity excludes the possibility of the formation of oligonuclear hydroxo-bridged complexes, as was assumed earlier,[5] thus the non-negligible base consumption taking place at pH > 10 can only be ascribed to the formation of the mononuclear hydroxo complex $[VOA_2(OH)]^{7-}$. The EPR results, however, strongly support the high stability of the bis(catecholato) binding for VO^{2+} at basic pH.

A remarkably different behaviour is observed with an increase in the ligand-to-metal molar ratio. Already at the 25:1 ratio a further species is identified in the EPR spectra over the range pH 4–9. This species is progressively favoured by increase in the Tiron excess, so that at a ratio of 100:1 it can be regarded as the only EPR-active species existing in solution (see Fig. 2). Also in this case the species can be assigned by spectral comparison with the vanadium(IV)–*o*-catechol system.[4] The EPR parameters, as well as the form of the spectrum, which consists of resonance lines broader than those of the mono- and bis-catecholato vanadium(IV) species indicating a higher molecular weight, assign the complex as a species analogous to tris(catecholato)vanadate(IV), namely $[VA_3]^{8-}$, where the bare vanadium(IV) ion is tris-chelated after displacement of the oxo group. Whatever the ligand-to-metal ratio, this species reaches a maximum in concentration over the range pH 4–9. In strongly basic solutions the complex equilibria revert toward formation of the $[VOA_2]^{6-}$ species. The extent to which VO^{2+} yields the tris complex depends not only on the ligand-to-metal ratio, but also on the total metal concentration. It can be observed that, when the molar ligand excess is kept constant, the fraction of tris-chelated vanadium(IV) becomes more significant with increasing metal concentration.

The complex-formation equilibria were also monitored by absorption spectroscopy. In accordance with the EPR results, in solutions with a ligand-to-metal molar ratio of 2:1, the

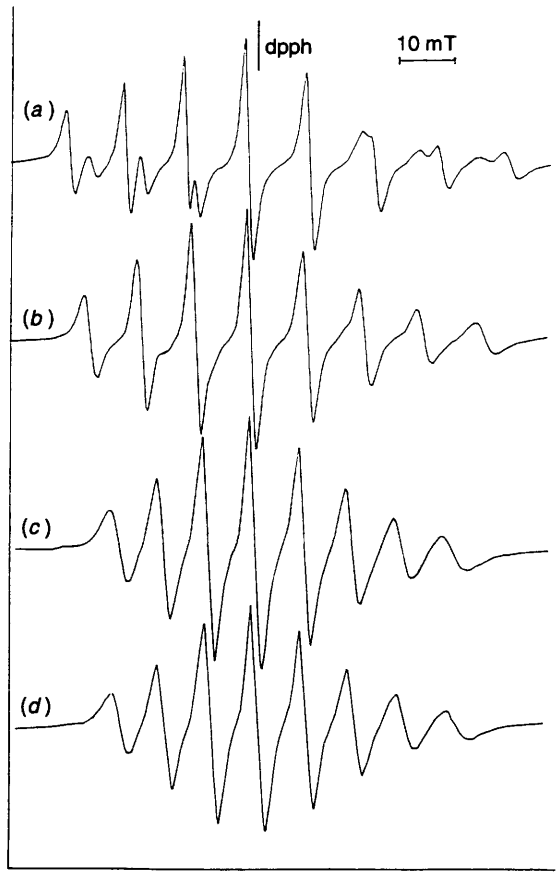


Fig. 1 X-Band EPR spectra recorded at room temperature on aqueous solutions of VO^{2+} (4×10^{-3} mol dm^{-3}) and Tiron at a ligand-to-metal molar ratio of 2:1 and varying pH: (*a*) 2.5, (*b*) 4.0, (*c*) 6.4 and (*d*) 12.0. dpph = Diphenylpicrylhydrazyl

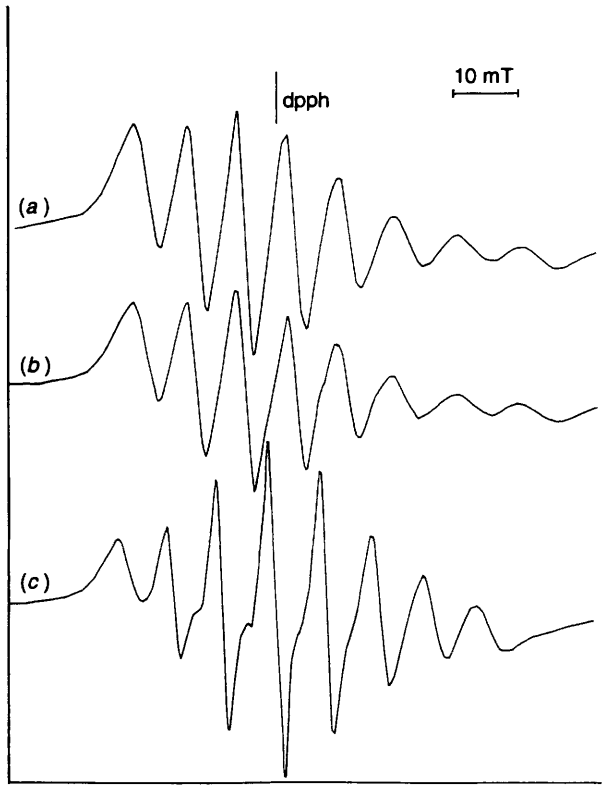


Fig. 2 X-Band EPR spectra recorded at room temperature on aqueous solutions of VO^{2+} (4×10^{-3} mol dm^{-3}) and Tiron at a ligand-to-metal molar ratio of 100:1 and varying pH: (*a*) 6.0, (*b*) 7.3 and (*c*) 11.8

$[VO(H_2O)_5]^{2+}$ was detected below pH 2.5 through d–d bands at 770 and 640 nm, with ε values of 17 and 8 dm^3 mol^{-1} cm^{-1}, respectively. In more basic solutions the mono- and bis-catecholato species were identified. The $[VOA]^{2-}$ complex exhibited bands at 525 and 705 nm, with ε values of 14 and 20 dm^3 mol^{-1} cm^{-1}, referred to the total metal concentration, at the maximum extent of formation. The ion $[VOA_2]^{6-}$ was characterised by bands at 530 and 650 nm with absorption coefficients of 35 and 61 dm^3 mol^{-1} cm^{-1}, in good agreement with earlier findings.[2,5] At higher ligand-to-metal molar ratios the formation of the tris complex was observed in solution through very intense absorption bands at 560 and 650 nm, which may be attributed to ligand-to-metal charge-transfer transitions.[2] Assuming that the intensity of these absorptions is a measure of the extent of formation of $[VA_3]^{8-}$, it was found that, in close agreement with the EPR results, the molar absorption coefficients referred to the total metal concentration increased with increasing ligand excess. Furthermore, if the ligand excess is kept constant, they also increase with increasing metal concentration. Electronic absorption spectra also support that the complex reaches a maximum in concentration over the range pH 4–8 beyond which $[VA_3]^{8-}$ is transformed into $[VOA_2]^{6-}$.

VO^{2+}–*Dopamine*.—Dopamine has a primary amino group in the side-chain of the catecholic aromatic nucleus. The analysis of the isotropic EPR spectra in aqueous solution indicates the formation of complexed species analogous to those of Tiron. These findings rule out any significant participation of the amino group in the metal co-ordination and substantiate that the ligand acts only as a simple *o*-catechol derivative. The monocatecholato complex is formed at pH values slightly greater than for the analogous Tiron species, substantiating the lower acidity of the phenolic hydroxyl groups. In neutral solution the bis(catecholato) complex is predominant. Owing to the negligible overlap between the formation of these complexes and the deprotonation of non-co-ordinated side-chain ammonium groups, the species can be assigned the stoichiometries $[VO(HA)]^+$ and $[VO(HA)_2]$, respectively, involving ligands bearing ammonium groups. The stepwise proton dissociation of $[VO(HA)_2]$ can be ascribed to the deprotonation of these ammonium groups. The pK values are 10.23 and 10.67, which closely correspond to the expected values (10.06 and 10.66), calculated from the microscopic dissociation constant pk_{12} (10.36)[16] of the free ligand and the statistical considerations [$pK_{VO(HA)_2} - pK_{VOA_2H} = 0.60$]. The reasonably good agreement between the measured and calculated deprotonation constants suggests that parallel hydroxo-complex formation processes in the basic pH range may have only a subordinate importance. Of course, the *O*,*O*′-co-ordinated complexes with different deprotonation extents cannot be differentiated by EPR spectroscopy. However, the $pK_{VO(HA)}$ value of 5.76, characteristic of deprotonation of the monocatecholato complex, can be ascribed only to the ionisation of a co-ordinated water molecule ($\log \beta_{1-1} = -6.07$ for the aqua ion).

As with Tiron, ligand-to-metal molar ratios greater than 2:1 favour the formation of the tris complex $[V(HA)_3]^+$ involving the ligand protonated at the amino group with an optimum formation range of pH 5–7. However, it appears that the tris complex of dopamine is more stable compared to that of Tiron. The EPR spectra show that, at a fixed ligand-to-metal molar ratio, the fraction of tris-chelated vanadium(IV) is higher in the presence of dopamine than in the corresponding Tiron system. A comparison of the electronic absorption spectra leads to the same conclusion. Even spectra recorded on solutions at 1×10^{-3} mol dm^{-3} oxovanadium and a ligand-to-metal molar ratio of 2:1 are affected by the presence of very small amounts of $[V(HA)_3]^+$, through characteristic bands at 595 and 660 nm, which become more intense with increasing either ligand excess or total metal concentration. The dependence of the intensity of

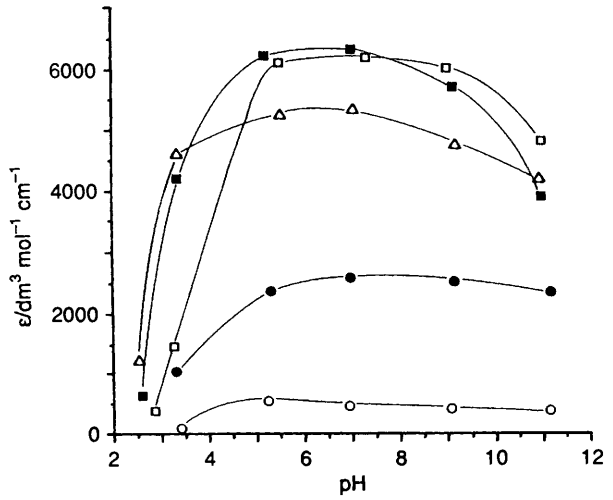


Fig. 3 Comparison of the intensity of the absorption maxima, as a function of pH, ligand-to-metal molar ratio (L:M) and total metal concentration (c/mol dm^{-3}), for vanadium(IV)–Tiron at 560 nm and –dopamine at 595 nm. Ligand = Tiron, (○) L:M = 100:1, c = 2 × 10^{-4}; (●) L:M = 50:1, c = 1 × 10^{-3}; (△) L:M = 100:1, c = 1 × 10^{-3}. Ligand = dopamine, (□) L:M = 50:1, c = 1 × 10^{-3}; (■) L:M = 100:1, c = 1 × 10^{-3}

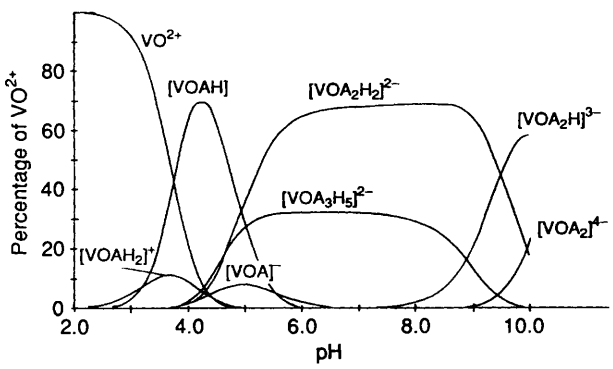


Fig. 4 Concentration distribution curves for the complexes formed in the VO^{2+}–L-dopa system as a function of pH; c_{VO} = 2 × 10^{-3} and c_{ligand} = 6 × 10^{-3} mol dm^{-3}

the absorption bands due to the tris complexes of Tiron and dopamine on pH, metal concentration and ligand-to-metal ratio is compared in Fig. 3. It can be observed that, at an oxovanadium(IV) concentration of 1 × 10^{-3} mol dm^{-3}, no significant increase in band intensity is observed if the dopamine-to-vanadium molar ratio is raised from 50:1 to 100:1, indicating that the major part of the metal ion is already tris-chelated. In comparison, an analogous variation of the ligand excess in the Tiron system is still able to promote the formation of the 1:3 complex.

VO^{2+}–*Epinephrine and –Norepinephrine*.—Epinephrine and norepinephrine behave similarly to dopamine and very similarly to each other. According to EPR spectra, metal complexation starts at slightly higher pH values than for Tiron and again involves only the catecholic moiety of the ligands. The species with 1:1 and 2:1 ligand-to-metal stoichiometry exhibit the same structure and follow the same distribution as in the corresponding dopamine system. The stepwise formation constants follow the same sequence as the basicity of the co-ordinating phenolate groups of the ligands (*cf.* Tables 1 and 2). Similarly, the stepwise deprotonation constants of the complex $[VO(HA)_2]$ also correspond well to the microscopic dissociation constants of the ammonium groups of the ligands (pk_{12} = 9.81 and 9.42 for epinephrine and norepinephrine, respectively). The tris complex of vanadium(IV) $[V(HA)_3]^+$, analogous to that of dopamine, predominates at high ligand excess. As reported,[23] the ligands precipitate in concentrated solutions at pH > 9. However, as shown by the EPR spectra, over the range pH 4–9 the tris complexes of epinephrine and norepinephrine are remarkably more stable than those of Tiron and less stable compared with those of dopamine. The same conclusion may be reached by analysis of electronic absorption spectra. For instance, at a metal concentration of 1 × 10^{-3} mol dm^{-3}, an increase in the ligand molar excess from 50:1 to 100:1 results in further formation of tris-chelated species (λ_{max} = 580 and 660 nm). Under the same conditions, no significant change in the intensity of the absorptions is observed in the vanadium(IV)–dopamine system.

VO^{2+}–L-dopa.—The compound L-dopa is well known for its ambidentate chelating behaviour, being a potential donor through either the catecholate or α-amino acid sites. The EPR spectra show that with oxovanadium(IV) the catecholate type of bonding is strongly favoured and monomeric 1:1 and 1:2 *O,O'*-co-ordinated species, namely [VO(HA)], $[VOA]^-$, $[VO(HA)_2]^{2-}$, $[VOA_2H]^{3-}$ and $[VOA_2]^{4-}$, where the undissociated protons are attributed to the ammonium groups, are observed. From data in Table 2 and speciation curves in Fig. 4, besides these catecholate-type species, a doubly deprotonated 1:1 complex $[VOAH_2]^+$ could also be detected by potentiometry in the acidic pH range. When the formation of this species was assumed, the fitting of the titration curves at a ligand-to-metal molar ratio of 1:1 improved by about 20%. This stoichiometry can be ascribed to an amino acid-type (NH_2, CO_2^-) co-ordination of the ligand in which the diphenolic site is not deprotonated.

The ligand is less soluble compared with the others examined here, so that only solutions with VO^{2+} concentrations of 2 × 10^{-3} or 1 × 10^{-3} mol dm^{-3} and ligand-to-metal molar ratios up to 25:1 were suitable to follow the formation of the tris complexes over the whole measurable pH range. However, under these conditions, both EPR and electronic absorption spectra (λ_{max} = 600 and 660 nm) substantiated that the extent of formation for the tris-chelated complex $[V(HA)_3]^{2-}$ is comparable with that observed for the norepinephrine and epinephrine systems.

A more general comparison shows that, for solutions of the same concentration, ligand excess and pH values, the amount of tris(catecholato)vanadate(IV) complex decreases in the order: dopamine > epinephrine, norepinephrine > L-dopa > *o*-catechol > 3,4-dihydroxybenzoic acid > Tiron (see the comparison of EPR spectra in Fig. 5). This trend is also seen in the whole range pH 4–11 and it may be considered as measuring the different tendencies of the ligands to yield tris-chelated species.

Conclusion

Spectroscopic and potentiometric data clearly demonstrate that catecholamines and the catecholamino acid L-dopa co-ordinate to VO^{2+} *via* their catecholate moiety. The co-ordination ability of the side-chain donors is negligible, except for a slight indication of *N,O*-bond formation in the L-dopa system at acidic pH. The results confirm that the formation of tris-chelated complexes of vanadium(IV) is a general reaction in all the catecholic systems. In all of such species the trigonal-prismatic distortion imposed by the ligand bite is responsible for the switch of the d_{xy} into the d_{z^2} ground state for the metal ion, as supported by the distinctive anisotropic EPR parameters. For instance, the following axial parameters (no evidence of rhombic anisotropy was obtained at least at the X-band frequency) were measured, using usual methods,[24] for the L-dopa complexes at 140 K: [VO(HA)], $g_{\|}$ = 1.943, $g_{\perp}$ = 1.985, $A_{\|}$ = 169 × 10^{-4} and $A_{\perp}$ = 69 × 10^{-4} cm^{-1}; $[VO(HA)_2]^{2-}$, $g_{\|}$ = 1.953, $g_{\perp}$ = 1.995, $A_{\|}$ = 155 × 10^{-4} and $A_{\perp}$ = 57 × 10^{-4} cm^{-1}. The tris complex $[V(HA)_3]^{2-}$ exhibits $g_{\perp}$ = 1.943 and

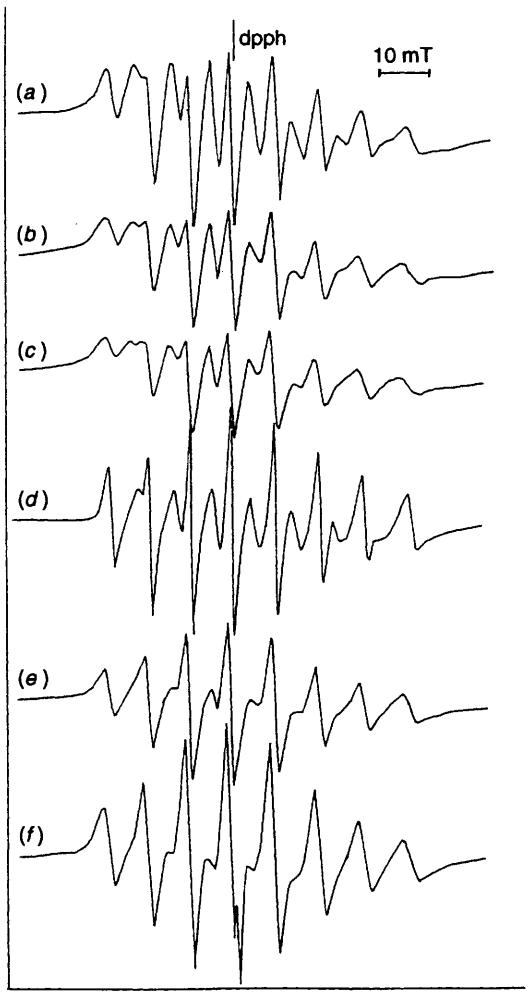


Fig. 5 X-Band EPR spectra recorded at room temperature on aqueous solutions of VO^{2+} (2×10^{-3} mol dm^{-3}) and catecholic ligands at a ligand-to-metal molar ratio of 25:1 and pH 7: (*a*) dopamine, (*b*) epinephrine, (*c*) L-dopa, (*d*) *o*-catechol, (*e*) 3,4-dihydroxybenzoic acid and (*f*) Tiron

$A_{\perp} = 109 \times 10^{-4}$ cm^{-1}, whereas $g_{\parallel}$ is *ca.* 2.00 and the magnitude of $A_{\parallel}$ is too small to be measured (see Fig. 6).

Approximate stability constants could be calculated for the tris complexes from the pH-metric titration data, although with a larger uncertainty than is usual. In these calculations the tris complex had also to be regarded as an association of VO^{2+}, A and H^+, thus it was defined as VOA_3H_5 or $[V(HA)_3]\cdot H_2O$. The formation of the tris complex takes place in the acidic pH range according to the process $[VO(HA)_2] + H_2(HA) \rightleftharpoons [V(HA)_3]\cdot H_2O$ [$H_2(HA)$ is the ligand protonated at both catecholic site and amino group; charges are omitted] without consumption or liberation of protons. Its decomposition in the basic pH range, where the first phenolic hydroxyl of the free ligand is already, at least partially, deprotonated, will result in an increase in acidity: $[V(HA)_3] + H_2O \rightleftharpoons [VO(HA)_2] + H(HA) + H^+$ [H(HA) is the ligand protonated at the NH_2 group and singly deprotonated at the catecholic moiety; charges are omitted]. However, these pH-metrically determined stability constants can be regarded only as tentative values, because the tris-complex formation in the acidic pH range (where it is not accompanied by any change in the pH of the solution) is fairly slow. For this reason, their formation during pH-metric titrations may have reached only 'quasi-equilibrium' (pH equilibrium but not formation equilibrium).[5] The waiting time at each titration point was limited by the oxygen sensitivity of the ligands. Despite these uncertainties, the formation constants show reasonably good agreement with that obtained

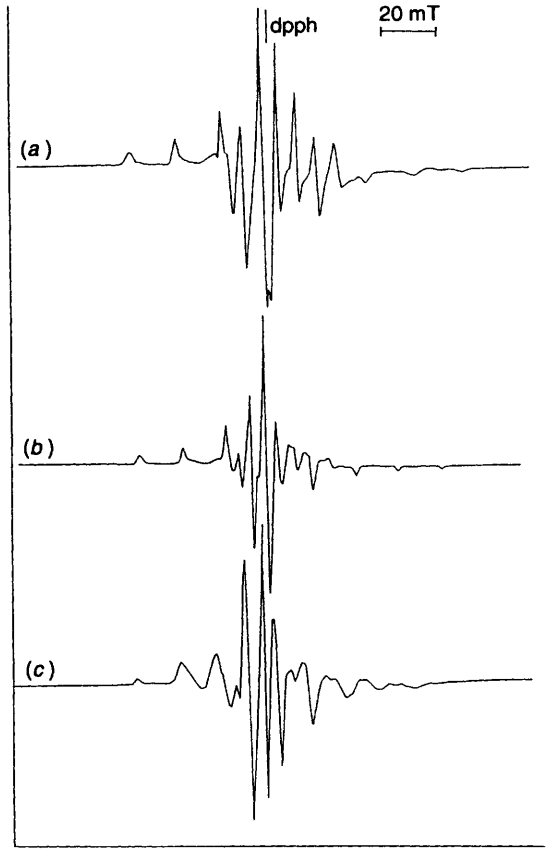


Fig. 6 X-Band EPR spectra recorded at 140 K on aqueous solutions of VO^{2+} (2×10^{-3} mol dm^{-3}) and L-dopa at varying ligand-to-metal molar ratio (L:M) and pH values: (*a*) L:M = 2:1, pH 4.5; (*b*) L:M = 2:1, pH 9.8; (*c*) L:M = 50:1, pH 7.0

by a spectrophotometric method for the respective tris complex of Tiron (log K^* = 2.03).[5]

A well defined trend was observed when comparing the different ability of the ligands to form the tris complex. Several factors may be important in determining this trend. For instance, electrostatic factors seem to be basically important, as a meaningful correlation can be found between the tendency to tris-complex formation and the charge of the 1:2 complex (6− for Tiron, 4− for 3,4-dihydroxybenzoic acid, 2− for *o*-catechol or L-dopa and 0 for epinephrine, norepinephrine or dopamine) or the net charge of the reacting partners, the 1:2 complex and ligand (8− for Tiron, 5− for 3,4-dihydroxybenzoic acid, 2− for *o*-catechol or L-dopa and 1+ for epinephrine, norepinephrine or dopamine). However, such an assumption does not explain the differences in stability of the tris complexes of epinephrine, norepinephrine and dopamine. With these ligands, besides electrostatic effects, another factor, the basicity of the phenolate oxygens, may be important, as a parallel change can be observed in the order of pk_1 values characteristic of the dissociation of the first phenolic hydroxyl groups and the stability of the tris complexes.

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