DALTON
FULL PAPER

A kinetic study of the reaction between noradrenaline and iron(III): an example of parallel inner- and outer-sphere electron transfer

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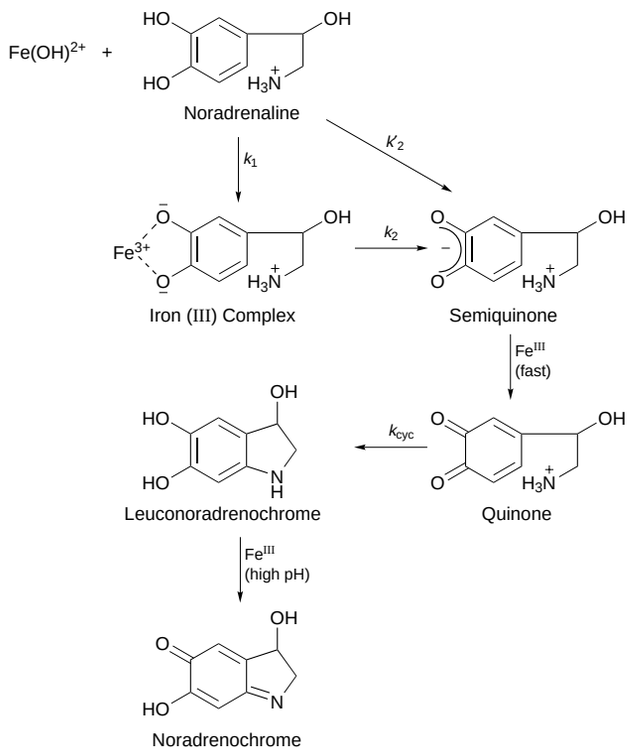
In anaerobic acid solution noradrenaline [norepinephrine, 4-(2-amino-1-hydroxyethyl)benzene-1,2-diol, H_2LH^+ (in which the phenolic protons are written on the left of L)] reacts with iron(III) [in the form of $Fe(OH)^{2+}$] to yield iron(II) and the semiquinone form of noradrenaline which is in turn oxidised rapidly by more iron(III) to 'noradrenoquinone'. This reaction proceeds both directly (*i.e. via* an 'outer-sphere' reaction) and after prior formation of the complex $Fe(LH)^{2+}$ which then decomposes *via* intramolecular electron transfer. [The observed rate of formation of the complex (monitored at 714 nm) is faster than the rate of its decomposition by a factor of about 200.] The quinone then cyclises by an intramolecular Michael addition giving the (UV transparent) leuconoradrenochrome (indoline-3,5,6-triol), which is able to react with iron(III) at high pH to give noradrenochrome (3,5-dihydro-3,6-dihydroxy-2*H*-indol-5-one). At lower pH values the presence of chloride ions shows a marked effect on the rate of complex formation because the species $FeCl^{2+}$ is also able to react with noradrenaline to form the complex although chloride is not involved in the reverse reaction. The stability constant for the formation of $FeCl^{2+}$ (K_1^{Cl}) was found to be 35, *i.e.* $\log K_1^{Cl} = 1.54$ (identical to the value obtained from previous work with dopamine). The ring-closure reaction was studied by following the rate of quinone decomposition monitored at 380 nm, and a mechanism for this cyclisation is proposed. The following rate constants have been evaluated: (i) for the reversible formation of the iron–noradrenaline complex [*via* $Fe(OH)^{2+} + H_2LH^+$] $k_1 = 2170 \pm 20$ dm^3 mol^{-1} s^{-1} and $k_{-1} = 21 \pm 2$ dm^3 mol^{-1} s^{-1}, from which the stability constant of the $Fe(LH)^{2+}$ complex has been calculated ($\log K_1^M = 21.2$), (ii) rate of formation of the complex *via* $FeCl^{2+} + H_2LH^+$, $k_{Cl} = 48 \pm 3$ dm^3 mol^{-1} s^{-1}, (iii) rate of decomposition of the complex $Fe(HLH)^{3+}$, $k_2 = 2.6 \pm 0.1$ s^{-1} [protonation constant for $Fe(LH)^{2+}$, $K_M^H = 34 \pm 1$ dm^3 mol^{-1}], (iv) rate of outer-sphere redox reaction, $k_2' = 100 \pm 2$ dm^3 mol^{-1} s^{-1}, (v) rate of indole formation (ring-closure reaction), $k_{cyc} = 1400 \pm 20$ s^{-1} (for quinone) and $k_{cyc}^H = (2.0 \pm 0.1) \times 10^5$ s^{-1} (for protonated quinone). All measurements were carried out at 25.0 °C in solutions of ionic strength 0.10 mol dm^{-3} (KNO_3 serving as inert electrolyte).

The interactions between iron and the catecholamines are of special interest because of their involvement in the progression of Parkinson's disease. In this respect 6-hydroxydopamine [5-(2-aminoethyl)benzene-1,2,4-triol] has attracted special attention because, unlike most of the catecholamine family, no complex with iron(III) can be detected, but it reacts directly[1] with iron(III) to form iron(II) and a semiquinone (*i.e.* by an 'outer-sphere' electron-transfer reaction). This is almost certainly the reason that it is capable of extracting iron from ferritin.[2] Most catecholamines (*e.g.* dopa [3-(3,4-dihydroxyphenyl)alanine],[3] dopamine [4-(2-aminoethyl)benzene-1,2-diol],[4] and adrenaline {4-[1-hydroxy-2-(methylamino)ethyl]benzene-1,2-diol}[5]), on the other hand, initially form a complex with iron(III) and the redox reaction then proceeds *via* internal electron transfer within the complex. This difference in redox behaviour is certainly related to the one-electron redox potentials of the species involved, and this would seem to be confirmed by the present study on noradrenaline which reacts with iron(III) *via* both reactions (*i.e.* by parallel 'inner-sphere' and 'outer-sphere' electron-transfer reactions). However, it must be admitted that the values of the one-electron redox potentials reported by Steenken and Neta[6] do not fully agree with this conclusion, but the very different conditions under which they were obtained (at high pH in glycol) makes their relevance uncertain.

When iron(III) is added to an acidic solution of noradrenaline [norepinephrine, 4-(2-amino-1-hydroxyethyl)benzene-1,2-diol] a dark green colour appears which rapidly fades and can be restored by the addition of excess iron(II). In the present case this green colour is due to the formation of a 1:1 complex between iron(III) and the dihydroxy function of noradrenaline, which in turn can be protonated at one of the co-ordinated oxygen atoms[7] at which point internal electron transfer takes place. As stated above, an exception to this rule is found[1,8] with 6-hydroxydopamine which does not produce a coloured complex but reacts directly with the iron(III) to form the *p*-quinone (*via* the semiquinones). It is particularly interesting, therefore, to see from the present work that noradrenaline exhibits a relatively small contribution from the 'outer-sphere' reaction as well as the more usual route *via* complex formation. However the final product of the redox process at low pH is the yellow-green noradrenoquinone [formed by rapid reaction of the semiquinone with another iron(III)]. This subsequently cyclises by an intramolecular Michael condensation to form the UV transparent leuconoradrenochrome (indoline-3,5,6-triol). This can be further oxidised to yield the pink noradrenochrome (3,5-dihydro-3,6-dihydroxy-2*H*-indol-5-one), although this step was not followed in the present study because it only takes place at a higher pH than that which we have employed. These steps are summarised in Scheme 1.

Experimental

Solutions of the required pH were made up from deoxygenated stock solutions of noradrenaline (supplied as tartrate and converted to nitrate by use of an ion-exchange column) and of iron(III) (as nitrate nonahydrate, Merck, *p.a.*) that contained a calculated amount of HNO_3 and sufficient KNO_3 to maintain the final ionic strength at 0.100 mol dm^{-3}. Experiments were also carried out with solutions containing KCl in order to investigate the effect of chloride ions on the reaction. The pH



Scheme 1 Overall route of oxidation of noradrenaline

was measured immediately after each kinetic run with a WTW 521 pH meter and $[H^+]$ was calculated by the empirical relationship[9] $[H^+] = 10^{-[(pH - 0.131)/0.982]}$. The concentrations of iron(III) used were sufficiently low to ensure that any changes in pH during reaction were negligible.

Kinetic stopped-flow techniques using absorption within the visible and near-UV region were carried out with a Bio-sequential SX-17MV (sequential stopped-flow ASVD spectrophotometer), supplied by Applied Photophysics, Ltd. (London). The formation of the complex was followed at 714 nm, while the formation and disappearance of the quinone were monitored at 380 nm. All kinetic runs were performed with noradrenaline in great excess over iron(III) in order to maintain pseudo (first)-order kinetics.

Results and Discussion

Complex formation in nitrate media

Above pH *ca.* 2 the rate of formation of the complex formed between iron(III) and noradrenaline is accurately first order in both $[Fe]_T$ and $[L]_T$, but at lower pH the rate varies with, but is not first order in $[L]_T$. This behaviour has been found for the closely related catecholamines, adrenaline, dopamine and dopa,[5,10] and arises from reversibility of the reaction.

Typical first-order rate constants, k_1^{obs}, for complex formation are given in Table 1 and presented graphically in Fig. 1 which shows that the rate passes through a distinct minimum at pH *ca.* 2. Bearing in mind that $Fe(OH)^{2+}$ is far more reactive than Fe^{3+} and has a protonation constant (K^{FeOH}) equal to[3] 660 $dm^3\ mol^{-1}$ [equation (1)], one can readily explain the acceleration with decreasing $[H^+]$. To explain the opposite effect at lower pH and the fact that the rate of complex formation becomes less than first-order dependent on the total noradrenaline concentration, $[L]_T$, it is necessary to take reversibility into account. Therefore it is proposed that both complexes are formed according to reaction (2). Over the pH range under

$$Fe(OH)^{2+} + H^+ \rightleftharpoons Fe^{3+}{}_{(aq)}; \quad \log K^{FeOH} = 2.82 \quad (1)$$

Table 1 Typical values of k_1^{obs} $\{[Fe]_T = (1.0 - 5.0) \times 10^{-4}\ mol\ dm^{-3}\}$

pH	$10^3[H^+]$/ mol dm^{-3}	$10^3[L]_T$/ mol dm^{-3}	k_1^{obs}/ s^{-1}	$k_1^{obs}[H^+]^{-1}$/ $dm^3\ mol^{-1}\ s^{-1}$	$[L]_T[H^+]^{-2}$/ $dm^3\ mol^{-1}$
1.20	81.5	13.80	2.38	29.2	2.07
1.22	77.8	6.90	1.97	25.3	1.14
1.22	77.8	10.35	2.17	27.9	1.71
1.24	74.2	5.00	1.72	23.2	0.91
1.25	72.5	10.00	1.95	26.9	1.90
1.27	69.2	10.00	1.80	26.0	2.10
1.27	69.2	5.00	1.61	23.3	1.04
1.27	69.2	7.50	1.79	25.9	1.57
1.28	67.6	7.50	1.64	24.3	1.64
1.35	57.4	13.80	2.07	36.1	4.20
1.36	5.6	6.90	1.62	28.9	2.19
1.36	5.6	10.35	1.81	32.3	3.30
1.52	38.5	10.00	1.73	45.0	6.75
1.53	37.6	7.50	1.45	38.6	5.31
1.53	37.6	10.00	1.68	44.7	7.07
1.55	35.9	7.50	1.44	40.1	5.82
1.58	33.5	5.00	1.21	36.1	4.46
1.58	33.5	10.00	1.60	47.8	8.91
1.64	2.91	3.50	0.72		
1.66	2.77	5.00	0.90		
2.00	1.25	5.00	0.74		
2.28	0.648	5.00	1.00		
2.50	0.387	5.00	1.47		
3.15	0.084	5.00	4.20		
3.21	0.073	5.00	4.43		

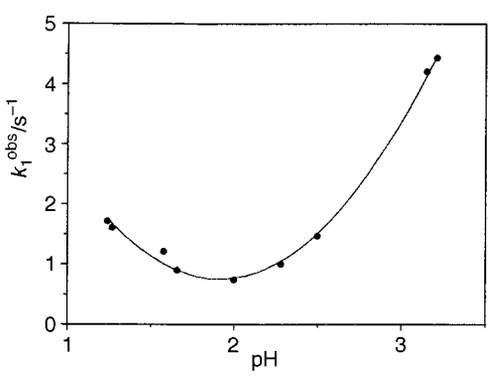


Fig. 1 Variation with pH of the observed rate constants, k_1^{obs}, for the formation of the iron(III)–dopamine complex ($[Fe]_T = 2.5 \times 10^{-4}$ mol dm^{-3}, $[L]_T = 5 \times 10^{-3}$ mol dm^{-3}). Data from Table 1

$$Fe(OH)^{2+} + H_2LH^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Fe(LH)^{2+} + H_3O^+ \quad [\text{or } Fe(HLH)^{3+} + H_2O] \quad (2)$$

consideration, the total uncomplexed iron(III) is given by equation (3), and the observed rate law can be written as in

$$[Fe]_T = [Fe^{3+}] + [Fe(OH)^{2+}] \quad (3)$$

equation (4), and hence in terms of reaction (2) the rate expression is given by equation (5). By assuming the equilibrium condition (6) and because: (i) $[Fe(LH)^{2+}]_{eq} = ([Fe]_{T,0} - [Fe]_{T,eq})$

$$d[\text{coloured complex}]/dt = k_1^{obs}([Fe]_{T,0} - [Fe]_{T,eq}) \quad (4)$$

$$= k_1[Fe(OH)^{2+}][H_2LH^+] - k_{-1}[Fe(LH)^{2+}][H^+] \quad (5)$$

$$k_1/k_{-1} = [Fe(LH)^{2+}]_{eq}[H^+]/[Fe(OH)^{2+}]_{eq}[H_2LH^+] \quad (6)$$

and (ii) noradrenaline is used in great excess giving, since it is fully protonated at these pH values, $[H_2LH^+] \approx [L]_T$, (iii) $K^{FeOH}[H^+] \gg 1$ at these low pH values.

Table 2 Variation of k_1^{obs} with $[Cl^-]$ (pH 1.10, $[L]_T = 0.055$ mol dm^{-3})

$[Cl^-]$/mol dm^{-3}	0.055	0.065	0.075	0.085	0.095
k_1^{obs}/s^{-1}	2.87	3.45	3.85	4.32	4.82

Table 3 Typical values of k_{Cl}^{obs}

$[Cl^-]$/ mol dm^{-3}	pH	$10^2[H^+]$/ mol dm^{-3}	$10^3[L]_T$/ mol dm^{-3}	k_{Cl}^{obs}/s^{-1}
0.0075	1.12	9.84	6.90	3.04
	1.12	9.84	10.35	3.21
	1.12	9.84	13.80	3.41
	1.21	7.97	6.90	2.57
	1.21	7.97	10.35	2.80
	1.21	7.97	13.80	2.98
0.025	1.15	8.98	6.90	4.06
	1.15	8.98	13.80	4.60
	1.15	8.98	17.30	4.89
	1.30	5.75	10.35	3.17
	1.30	5.75	13.80	3.47
	1.30	5.75	17.30	3.69
0.0384	1.58	5.35	6.90	2.50
	1.59	3.27	10.35	3.18
	1.56	3.51	12.40	3.47
	1.87	1.59	6.90	2.61
	1.87	1.59	10.35	3.51
	1.87	1.59	12.40	3.99

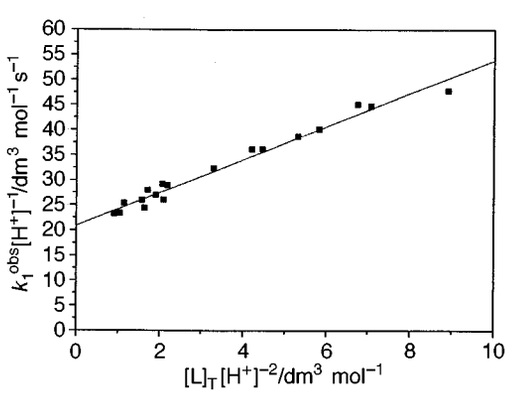


Fig. 2 Plot of $k_1^{obs}/[H^+]$ *vs.* $[L]_T/[H^+]^2$ for values below pH 1.6 (data from Table 1)

Therefore the equilibrium condition (6) reduces to (7). Inserting this and the above assumptions into the rate law leads, after comparison with the observed rate law (4), to equation (8).

$$[Fe]_{T,eq} = k_{-1}K^{FeOH}[H^+]^2[Fe]_{T,0}/(k_1[L]_T + k_{-1}K^{FeOH}[H^+]^2) \quad (7)$$

$$k_1^{obs}K^{FeOH}[H^+] = k_1[L]_T + k_{-1}K^{FeOH}[H^+]^2 \quad (8)$$

Thus a plot of $k_1^{obs}/[H^+]$ *vs.* $[L]_T/[H^+]^2$ should be linear with intercept corresponding to k_{-1} and a slope of k_1/K^{FeOH}. This is illustrated in Fig. 2 (Table 1) and, using $K^{FeOH} = 660$ dm^3 mol^{-1}, yields the results $k_1 = 2170 \pm 20$ dm^3 mol^{-1} s^{-1} and $k_{-1} = 21 \pm 3$ dm^3 mol^{-1} s^{-1}. The ratio k_1/k_{-1} thus enables the stability constant, K_1^M, of the $Fe(LH)^{2+}$ complex $[Fe^{3+} + LH^- \overset{K_1^M}{\longleftrightarrow} Fe(LH)^{2+}]$ to be calculated from the relationship $K_1^M = k_1\beta_2^H/k_{-1}K^{FeOH}$ (where β_2^H is the microconstant for the protonation of the phenolic groups[11] of the noradrenaline). A value of log $K_1^M = 21.2$ was obtained.

Complex formation in the presence of chloride

At pH values below 2 the presence of chloride ions has a marked effect on the rate of complex formation. The results in Table 2 show clearly that the rate constant, k_1^{obs}, is proportional to $[Cl^-]$, and Table 3 reports a series of measurements made at varying $[L]_T$, $[Cl^-]$ and $[H^+]$ values.

When equation (8) is applied to data obtained in chloride media it is seen that the effect on the reverse reaction, represented by k_{-1}, is directly proportional to $[Cl^-]$, whereas the effect on the forward reaction, k_1, is much less and decreases with increase in pH.

These effects can be explained by assuming that the species $FeCl^{2+}$ is also able to react with H_2LH^+ [equation (9)], which is

$$FeCl^{2+} + H_2LH^+ \xrightarrow{k_{Cl}} FeLH^{2+} + Cl^- + 2H^+ \quad (9)$$

predominantly reversible only *via* the non-chloride route (2). Allowance must also be made for the fact that $[Fe]_T$ must now be written as in equation (10) which reduces to (11) since it is

$$[Fe]_T = [Fe^{3+}] + [FeCl^{2+}] + [Fe(OH)^{2+}] \quad (10)$$

$$[Fe]_T = [Fe^{3+}] + [FeCl^{2+}] = [Fe^{3+}](1 + K_1^{Cl}[Cl^-]) \quad (11)$$

possible to neglect the value of $[Fe(OH)^{2+}]$ with respect to $[FeCl^{2+}]$ and in which K_1^{Cl} is the formation constant for $FeCl^{2+}$. Thus, in the presence of chloride, equation (5) must be replaced by (12). This leads to equation (8) being replaced by (13)† in

$$d[\text{coloured complex}]/dt = k_1[Fe(OH)^{2+}][H_2LH^+] - k_{-1}[Fe(LH)^{2+}][H^+] + k_{Cl}[FeCl^{2+}][H_2LH^+] \quad (12)$$

$$k_{Cl}^{obs} = (k_1 + k_{Cl}K^{FeOH}K_1^{Cl}[Cl^-][H^+])[L]_T/K^{FeOH}[H^+] + k_{-1}^{Cl}[H^+] \quad (13)$$

which $k_{-1}^{Cl} = k_{-1}(1 + K_1^{Cl}[Cl^-])$. Thus plotting $k_{Cl}^{obs}/[H^+]$ *vs.* $[L]_T/[H^+]^2$ yields $k_{-1}^{Cl} = k_{-1}(1 + K_1^{Cl}[Cl^-])$ as intercept and a slope dependent on $[H^+]$ and $[Cl^-]$ as observed (above).

Plotting k_{Cl}^{obs} *vs.* $[L]_T$ for sets of results at constant $[H^+]$ and $[Cl^-]$ yields straight lines with intercepts equal to $k_{-1}^{Cl}[H^+]$ and slopes of $\{(k_1 + k_{Cl}K^{FeOH}K_1^{Cl}[Cl^-][H^+])/K^{FeOH}[H^+]\}$ [see equation (13)]. From the slopes derived in this way we obtained a value of $k_{Cl} = 48 \pm 3$ dm^3 mol^{-1} s^{-1} [compare this with the corresponding value of 43 ± 2 dm^3 mol^{-1} s^{-1} obtained for the iron(III)–dopamine system].

Complex formation in the presence of bromide

Unlike chloride ions, the presence of bromide ions had no effect on the rate of reaction. Although this could be ascribed to $FeBr^{2+}$ being non-reactive, the concentration of this species is not detectable (*via* speciation effects) and so the lack of reactivity could equally well be a result of too low a concentration.

Decomposition (electron transfer) step

At constant $[H^+]$ and $[L]_T$ the rate of decomposition is given by equation (14), and some typical values of k_2^{obs} for pH < 2 are

$$-d[\text{coloured complex}]/dt = k_2^{obs}[Fe]_T \quad (14)$$

given in Table 4. Plotting $1/k_2^{obs}$ *vs.* $[H^+]$ for the values obtained at relatively low noradrenaline concentrations (Table 4) yields a series of straight lines with a common intercept of 70 (Fig. 3),

† Unfortunately, in ref. 12, the corresponding equation is incorrectly quoted; the factor $(1 + K_1^{Cl}[Cl^-])$ should have been omitted since it had been incorporated in k_{-1}^{Cl} and k_{Cl}^{obs}. The corrected value of k_{Cl} for dopamine is therefore 43 ± 2 dm^3 mol^{-1} s^{-1}.

Table 4 Typical values of k_2^{obs}

pH	$10^2[H^+]$/ mol dm^{-3}	$10^3k_2^{obs}$/ s^{-1}	$10^3[L]_T$/ mol dm^{-3}
(*a*) Results at low noradrenaline concentrations			
1.19	8.35	2.45	3.5
1.35	5.74	3.25	3.5
1.64	2.91	5.02	3.5
1.24	7.42	3.81	5.0
1.37	5.47	5.24	5.0
1.66	2.77	7.08	5.0
1.11	10.07	3.98	7.5
1.23	7.60	4.87	7.5
1.43	4.76	6.62	7.5
(*b*) Results at higher noradrenaline concentrations			
1.31	6.3	1.8	10
1.46	4.5	1.6	10
1.67	2.7	1.6	10
1.31	6.3	2.2	15
1.46	4.5	2.1	15
1.67	2.7	2.0	15
1.31	6.3	3.3	25
1.46	4.5	3.1	25
1.67	2.7	2.8	25

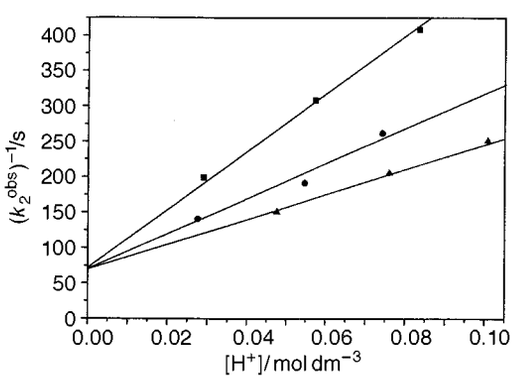


Fig. 3 Plot of $1/k_2^{obs}$ *vs.* [H$^+$] for 'low' $[L]_T$ values (Table 4): $10^3[L]_T$ = 3.50 (■), 5.00 (●), 7.50 (▲) mol dm^{-3}

the slopes of which are directly proportional to $[L]_T$. This can be expressed by equation (15) and can be shown to arise from

$$k_2^{obs} = [L]_T/(13.2[H^+] + 70[L]_T) \quad (15)$$

the reaction of $Fe(OH)^{2+}$ with H_2LH^+ to form the semiquinone [equation (16)], and since this is also the mode of formation

$$-d[Fe]_T/dt = k_2'[Fe(OH)^{2+}][H_2LH^+] \quad (16)$$

of the complex (above) this implies an 'outer-sphere' redox reaction. This has not been observed for any of the other catecholamines with the exception of 6-hydroxydopamine[1,8] which reacts solely by this route (*i.e.* there is no evidence for complex formation). Under these conditions of low pH and noting that complex formation had reached equilibrium we can write, to a good approximation equation (17), and inserting this result into

$$[Fe]_T = [Fe^{3+}] + [Fe(HLH)^{3+}] = [Fe^{3+}](\beta_2^H[H^+] + K_M^H K_1^M[L]_T)/\beta_2^H[H^+] \quad (17)$$

$$-d[Fe]_T/dt = k_2'\beta_2^H[L]_T[Fe]_T/K^{FeOH}(\beta_2^H[H^+] + K_M^H K_1^M[L]_T) \quad (18)$$

(16) yields (18). Noting also that the semiquinone formed reacts rapidly with another iron(III) to form the quinone, comparison of equation (18) with (14) yields (19). Using the experimental result equation (15) gives a value of $k_2' = 100 \pm 2$ dm^3 mol^{-1} s^{-1}.

Table 5 Typical rate constants of the formation of noradrenoquinone ($k_2'^{obs}$), followed at 380 nm

pH	$10^2[H^+]$/ mol dm^{-3}	$10^3[L]_T$/ mol dm^{-3}	$10^3[Fe]_T$/ mol dm^{-3}	$10^3\ k_2'^{obs}$/ s^{-1}
1.24	7.42	10.00	1.00	9.32
1.30	6.45	10.00	1.00	9.92
1.39	5.22	10.00	1.00	8.99
1.48	4.23	10.00	1.00	1.18
1.55	3.59	10.00	1.00	1.24
1.66	2.77	10.00	1.00	1.37

At relatively higher noradrenaline concentrations (Table 4), the contribution to the redox reaction of internal electron transfer within the protonated complex must be taken into account [equation (19)]. However, it is the total amount of

$$-d[Fe]_T/dt = k_2'[Fe(OH)^{2+}][H_2LH^+] + k_2[Fe(HLH)^{3+}] \quad (19)$$

complex, *i.e.* $[Fe(LH)^{2+}] + [Fe(HLH)^{3+}]$, that is being followed, and this is the coloured indicator for $[Fe]_T$ and thus (19) becomes (20) in which the term in k_2' is expressed in terms of

$$-d[Fe]_T/dt = \{[L]_T/(13.2[H^+] + 70[L]_T) + k_2K_M^H[H^+][L]_T/(1 + K_M^H[H^+])\}[Fe]_T \quad (20)$$

the experimental value (15) obtained above. Therefore, k_2^{obs} is given by equation (21) which enables k_2 and K_M^H to be calcu-

$$2k_2^{obs} = [L]_T/(6.6[H^+] + 35[L]_T) + k_2K_M^H[H^+][L]_T/(1 + K_M^H[H^+]) \quad (21)$$

lated from the data in Table 4. {Note that the differences required, namely $2k_2^{obs} - [L]_T/(6.6[H^+] + 35[L]_T)$, were too small in the case of $[L]_T = 0.01$ mol dm^{-3} and were therefore not used.} Values of $k_2 = 2.6 \pm 0.1$ s^{-1} and $K_M^H = 34 \pm 1$ dm^3 mol^{-1} were obtained. The rate constant, k_2, for the rate of decomposition of the complex is considerably higher than that obtained for dopamine[12] (0.23 s^{-1}) and this reflects the considerably greater stability of the dopamine complex against internal electron transfer followed by decomposition. The protonation constants, K_M^H, for the iron(III) complexes of all the catecholamines studied are, as would be expected, almost identical.

Formation of quinone

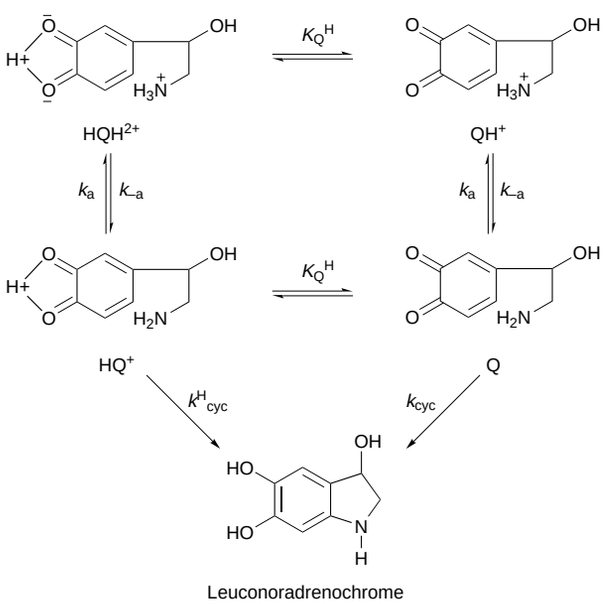
Table 5 contains some typical first-order rate constants ($k_2'^{obs}$) for quinone formation and it is readily ascertained that $k_2'^{obs}$ equals k_2^{obs} (*i.e.* the rate determining step in the formation of the quinone is the initial electron transfer in the complex). This implies that no information as to the actual rate of formation of the quinone from the semiquinone can be ascertained by this method of studying the reaction; this is the same result as was found for dopa,[10] and also confirms that the subsequent oxidation of the semiquinone is fast, as is to be expected for a radical–radical reaction.

Formation of the indole ring

The quinones of catecholamines such as noradrenaline spontaneously cyclise *via* an internal Michael addition to form the UV transparent leuconoradrenochrome (indoline-3,5,6-diol) (see Scheme 2). The kinetics of this cyclisation reaction for noradrenaline was followed by monitoring the quinone at 380 nm. This was possible by using iron(III) as an oxidant up to a pH of about 3, because the spectral absorption of the quinone exceeded that of the complex. At higher pH, however, the oxidation was performed using periodate as the oxidant, in order

Table 6 Typical values for the observed rate constant, k_3^{obs}, for indole formation (ring-closure reaction) following oxidation of the noradrenaline to the quinone

pH	Oxidant	log k_3^{obs}
0.93	Iron(III)	−3.55
1.58		−3.15
1.71		−3.19
1.73		−3.21
2.12		−2.80
3.74	Periodate	−2.20
4.90		−1.31
6.40		0.10
7.90		1.22
8.52		1.50
9.60		1.58



Leuconoradrenochrome

Scheme 2 Details of ring-closure reaction

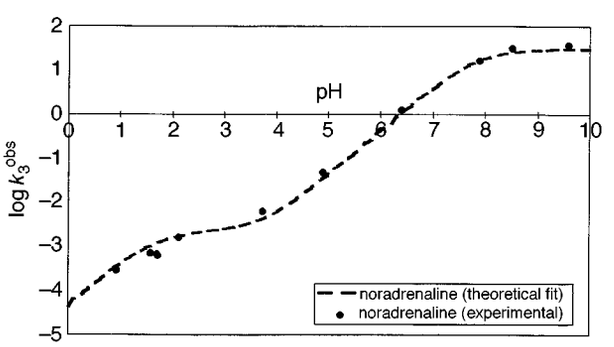


Fig. 4 Observed rate of cyclisation of noradrenoquinone, log k_3^{obs}, *vs.* pH (experimental points with the theoretical curve)

to avoid the interference of the iron(III) complexes which absorb at these higher pH values.

The rate of disappearance of the quinone follows the rate law (22) and typical results are summarised in Table 6 and illustrated in Fig. 4. The results strongly suggest that two quinone species are involved, one being protonated [equation (23)]. This must imply that protonation of the *quinone function* takes place at low pH. We further assume that deprotonation at the amino site is a requirement for cyclisation, and because the protonation constant of this functional group is very high [log $K_N^H = \log(k_a/k_{-a}) = 9.53$] this (deprotonation) step is relatively slow and must also be taken into account in formulating the reaction scheme. These ideas are summarised in Scheme 2.

$$-d[Q]_T/dt = k_3^{obs}[Q]_T \quad (22)$$

$$-d[Q]_T/dt = k_{cyc}[Q] + k_{cyc}^H[HQ^+] = (k_{cyc} + k_{cyc}^H K_Q^H[H^+])[Q] \quad (23)$$

The experimental results can be interpreted on the basis of this scheme and the associated postulates as follows. Under reaction conditions [Q] (or [HQ^+]) will reach a steady state, as shown in equations (24) and (25), but the total quinone concentration, $[Q]_T$, as given by equation (26), and the combination

$$d[Q]/dt = 0 = k_{-a}[QH^+] - k_a[Q][H^+] - k_{cyc}[Q] \quad (24)$$

$$[Q] = [QH^+]/(K_N^H[H^+] + k_{cyc}/k_{-a}) \quad (25)$$

$$[Q]_T = [QH^+] + [HQH^{2+}] = [QH^+](1 + K_Q^H[H^+]) \quad (26)$$

of equations (23), (25) and (26) taken in comparison with (22) leads to equation (27), in which $K_N^H = k_a/k_{-a}$ is the protonation microconstant for the amino group. The value of K_N^H is known[11] (log $K_N^H = 9.53$) and k_{-a} was obtained from the limiting value of k_3^{obs} at high pH [*i.e.* where $[H^+] \rightarrow 0$, see equation (27)] and has the value of 30.0 s^{-1}. Thus the rate constants for the cyclisation reaction and the protonation constant K_Q^H were readily obtained from equation (27): $k_{cyc} = 1400 \pm 20$ s^{-1} (for quinone), $k_{cyc}^H = (2.0 \pm 0.1) \times 10^5$ s^{-1} (for protonated quinone) and log $K_Q^H = 1.55$.

$$k_3^{obs} = (k_{cyc} + k_{cyc}^H K_Q^H[H^+])/\{(K_N^H[H^+] + k_{cyc}/k_{-a})(1 + K_Q^H[H^+])\} \quad (27)$$

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