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Comparison and thermodynamic studies on complexation of adrenaline with some metal ions in mixed solvents

Abstract: Two methods are described for the determination of stability constants of adrenaline with metal ions. In method A, the solution equilibria of Al(III) complex formed with bidentate ligand of adrenaline in acidic media at pH ranges of ≈3 to ≈5 have been studied in cosolvent systems at 15, 20, 25±0.1°C and I=0.2 mol.dm^{-3} sodium chloride, employed phosphate buffer, with spectrophotometric methods. Evidence for the 2:1 complex was not observed for aluminum. The stability constants of the complex was determined and the resulting free-energy changes were obtained. The protonation constants of adrenaline were determined under the above condition. The results are discussed in terms of the effect of solvent on protonation and complexation. In method B, the complexation reaction with Fe(III) ions has been studied in water in 15, 20, 25±0.1°C. The stoichiometry of the complex was found to be 1:2 (metal ion/ligand). The stoichiometry of Fe(III)-adrenaline was estimated by mole ratio and continuous variation methods and emphasized by the KINFIT program. The values of the thermodynamic parameters for complexation reaction was obtained from the temperature dependence of the stability constants.

Keywords: adrenaline; Al(III); complexation; dielectric; KINFIT; solvent; spectrophotometric.

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Introduction

Catechols can undergo a variety of chemical reactions. Catechols can act both as antioxidant, preventing lipid peroxidation, and as pro-oxidant damaging macromolecules such as DNA and proteins. Catechols can also destroy membrane functioning due to their redox cycling activity. In this section, some possible modes of action are summarized. In humans and mammals, catechols can occur as metabolites in the degradation of benzene or estrogens (Stone et al., 1995; Bolton et al., 1998) or as endogenous compounds, such as neurotransmitters and their precursors: adrenaline, noradrenaline, adrenaline and L-DOPA (L-3,4-dihydroxyphenylanaline). Additionally, catechols can be taken up in the form of tobacco smoke (as catechol, catechol semiquinones and polymerized catechols) (Oshima, 1965; Pryor et al., 1998) or as food components (e.g., catechol, adrenaline, caffeic acid, tea catechin) (Dustin, 1950; Hayakawa et al., 1997; Moran et al., 1997).

Adrenaline was the first hormone to be identified and was successfully synthesized in 1904. It is part of a family known as biogenic amines, which includes serotonin and histamine, among others. Epinephrine is the compound commonly also called adrenaline (ADR). Its specific compound group is the catecholamine group, which also includes norepinephrine and adrenaline. Sustained high levels of catecholamines in the blood are a good indicator of chronic stress.

In fact, when Al reaches the blood circulation and is not excreted by urine, it accumulates in tissues where it becomes strongly bound. Transferrin (Tf) seems to be the main Al binding protein in plasma (Trapp, 1983). Because it is only ca. 30% saturated with iron in normal serum, it still has a substantial binding capacity for chelating other trivalent metal ions, including Al. Also, it is now a well-accepted notion that iron is the most probable agent responsible for lipid peroxidative damage in the brain (Arai et al., 1987) and that aluminum can aggravate its action.

Catechols can form stable complexes with various di- and trivalent metal ions, the complexes with trivalent ions being the most stable. Aluminum is generally regarded as a toxic or detrimental element. Aluminum can form complexes with organic compounds, reducing the activity and subsequent toxicity of Al^{3+} in solution. As demonstrated in previous articles, equilibrium constants for Al complexation are therefore important for predicting levels available, Al^{3+} being the most important. For several years, chelating iron agents have aroused an increasing interest in many ways. Many bidentate ligands exhibit biological activity. Adrenaline is an iron(III) chelator.

This paper presents results of equilibrium and spectral studies of the interaction of adrenaline (ADR) with Al(III) in mixed solvent solutions, to show how solvents and solvent mixtures with different dielectric constants affect formation of such a complex. Also, the present paper describes the formation of the complex of Fe(III) with adrenaline.

Results and discussion

Protonation equilibria of the ligand

The protonation constants for ligand 1 mmol.dm^{-3} in water and mixed-solvent systems of ethanol and water were obtained from potentiometric titrations with 0.1 mol.dm^{-3} NaOH and employing a computer-programmed nonlinear least-squares method. Values of the constants obtained are listed in Table 1 and are in agreement with those obtained from the literature (pK_{a1}=8.89, pK_{a2}=10.41, pK_{a3}=13.1 at 25°C) (Kiss and Gergely, 1979a). We assume that deprotonation occurs in the following order with increasing pH: the paraphenolic group, the ammonium group and then the second OH group for adrenaline. The protonation constants are K_{1a} and K_{3a}. These values are listed in Table 1.

For the following general equilibriums:

$$H^+ + L^{2-} \xrightleftharpoons{K_{1a}} HL^- \qquad K_{1a} = [HL^-]/[H^+][L^{2-}] \tag{1}$$

$$H^+ + HL^- \xrightleftharpoons{K_{2a}} H_2L \qquad K_{2a} = [H_2L]/[H^+][HL^-] \tag{2}$$

$$H^+ + H_2L \xrightleftharpoons{K_{3a}} H_3L^+ \sim \; K_{3a} = [H_3L^+]/[H^+][H_2L] \tag{3}$$

The protonation constants are K_{1a}, K_{2a}, K_{3a}

Complexation Al(III) with adrenaline

Complexation equilibria of an Al(III) ion with adrenaline have been studied employing a technique based on the relationship of absorbance as a function of pH, $A=f$(pH). Absorbance measurements were carried out for solutions containing Al(III) and adrenaline with different pH ratios of ≈3 to ≈5. Considering that absorbance is a function of pH, the values of the molar absorptivities of Al(III), ε_0, at different wavelengths and various dielectric constants, are shown in Table 2.

x (molar fraction)	**log K_{1a}**	**log K_{3a}**
		t=15°C
1.000	8.68	13.15
0.95	8.71	13.16
0.90	8.75	13.16
0.85	8.79	13.18
0.80	8.81	13.19
0.75	8.83	13.20
		t=20°C
1.000	8.64	13.10
0.95	8.69	13.10
0.90	8.74	13.11
0.85	8.78	13.12
0.80	8.80	13.14
0.75	8.82	13.16
		t=25°C
1.000	8.61	13.05
0.95	8.66	13.06
0.90	8.70	13.07
0.85	8.73	13.09
0.80	8.75	13.10
0.75	8.80	13.11

Table 1 Average values of protonation adrenaline with standard deviations (0.01) in (x) water +(1-x) ethanol at different temperatures and I=0.2 mol.dm^{-3}.

In the equilibrium reaction of complex formation:

$$Al^{3+} + H_3L^+ \rightarrow Al(HL)^{2+} + 2H^+ \tag{4}$$

The formation constant of complex can be expressed as follows:

$$K^H_{Al(HL)^{2+}} = [Al(HL)^{2+}]\,[H+]^2/[Al^{3+}]\,[H_3L^+] \tag{5}$$

The absorbance at a wavelength is given by:

$$A = \varepsilon_0\,[Al^{3+}] + \varepsilon_1\,[H_3L^+] + \varepsilon_2\,[Al(HL)^{2+}] \tag{6}$$

where ε_0, ε_1, ε_2 are the molar absorptivities of the Al(III) ion and adrenaline and complex.

For the material balance:

$$[Al^{3+}] = C_{Al^{3+}} - [Al(HL)^{2+}] \tag{7}$$

$$[HL^-] = C_{H_3L^+} - [Al(HL)^{2+}] \tag{8}$$

where $C_{Al^{3+}}$ and $C_{H_3L^+}$ are the total concentrations of Al^{3+} and adrenaline. Thus, the equilibrium constant of formation reaction of complex can be expressed as follows:

$$\frac{A+\varepsilon_1 C_{Al^{3+}}+\varepsilon_1 C_{H_3L^+}}{C_{Al^{3+}}} = \varepsilon_2 + \frac{(\varepsilon_0+\varepsilon_1-\varepsilon_2)(-A+\varepsilon_1 C_{H_3L^+}+\varepsilon_0 C_{Al^{3+}})[H^+]^2}{C_{Al^{3+}}(A-\varepsilon_0 C_{Al^{3+}}-\varepsilon_2 C_{H_3L^+}+\varepsilon_0 C_{H_3L^+})K^H_{Al(HL)^{2+}}} \tag{9}$$

λ						λ (nm)
x	ε ($M^{-1}\times cm^{-1}$)	240	250	260	270	280
						t=15°C
1.000	ε_0	638	108	73	72	70
	ε_2	1425	1128	1121.6	783.16	652.73
0.95	ε_0	680	167	126	116	114
	ε_2	1432	1294.5	1126	790.68	702.21
0.9	ε_0	689	169	127	119	116
	ε_2	1498.5	1298.8	1216.3	805.01	720.85
0.85	ε_0	738	174	131	129	126
	ε_2	1520	1305.3	1292.2	860.07	730.93
0.8	ε_0	777	205	154	142	139
	ε_2	1612.9	1213.1	1312.6	903.78	736.35
0.75	ε_0	821	227	183	169	165
	ε_2	1656.4	1459	1418.8	1040.9	980.23
						t=20°C
1.000	ε_0	637	77	37	34	30
	ε_2	1435.3	1170	1132.3	755.63	626.12
0.95	ε_0	695	89	41	36	34
	ε_2	1445.4	1190.9	1139.9	773.94	708.27
0.9	ε_0	720	90	57	51	50
	ε_2	1528.8	1313.1	1225.4	839.76	760.88
0.85	ε_0	736	105	74	68	63
	ε_2	1617	1373.9	1292.7	876.22	771.46
0.8	ε_0	759	125	131	122	113
	ε_2	1664.8	1393.3	1319	878.1	781.86
0.75	ε_0	823	183	137	125	123
	ε_2	1728.7	1502.3	1322.6	1040.9	982.93
						t=25°C
1.000	ε_0	629	75	33	31	29
	ε_2	1475.6	1193.1	1168.8	733.62	608.16
0.95	ε_0	675	81	39	35	33
	ε_2	1479.4	1250.2	1170.7	753.7	682.75
0.9	ε_0	681	88	56	49	43
	ε_2	1554.7	1257	1247.4	823.18	746.35
0.85	ε_0	733	104	69	65	62
	ε_2	1612.9	1275.1	1257.1	865.76	754.3
0.8	ε_0	749	120	125	117	112
	ε_2	1651	1475.5	1325.9	1037	760.24
0.75	ε_0	802	181	132	123	115
	ε_2	1724	1517.1	1418.7	2024.8	993.69

Table 2 Values of molar absorptivities of Al ($\times 10^{-4}$ ε_0) and $Al(HL)^{2+}$($\times 10^{-4}$ ε_2) in (*x*) water +(1 - *x*) ethanol at different temperatures and I=0.2 mol.dm^{-3}.
x=molar fraction of water, ε_0, ε_2=molar absorptivities of metal and complex, respectively.

Considering that A is a function of pH, the values of molarity absorptivities are shown in Table 2. The values of were determined from the intercept of the straight line plots of $\left(A+\varepsilon_1 C_{Al^{3+}}+\varepsilon_1 C_{H_3L^+}\right)\Big/C_{Al^{3+}}$ (Y) against $\left(-A+\varepsilon_1 C_{H_3L^+}+\varepsilon_0 C_{Al^{3+}}\right)[H^+]^2\Big/C_{Al^{3+}}$ (X) and are shown in Table 3. From the intercept of the line ε_2 is calculated. The absorbance of $Al(HL)^{2+}$ at different pH and wavelengths in ethanol are listed in Table 2. The stability constant of $Al(HL)^{2+}$ complex is calculated by combining the protonation constants of adrenaline with the formation constants of complexes (Table 3).

$$KAl(HL)^{2+}=[Al(HL)^{2+}]/\ [Al^{3+}]\ [HL= K^{H}_{Al(HL)^{2+}}+K_{1a}K_{3a} \quad (10)$$

Enthalpy changes were obtained by plotting log K vs. $1/T$ (Figure 1). The variation of protonation constant or change in free energy with co-solvent content depends upon two factors, that is, electrostatic and non-electrostatic. Born's classical treatment holds good in accounting for the electrostatic contribution to the free energy

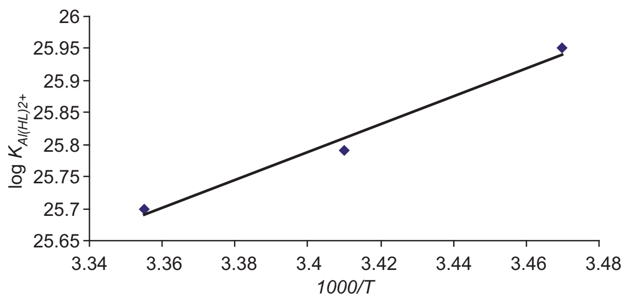

Figure 1 Plot of log $K_{Al(HL)^{2+}}$ vs. $1/T$ for (x) water +(1 - x) ethanol.

change (Schaefer and Karplus, 1996; Bashford and Case, 2000).

$$\Delta G = \frac{-q^2 e^2}{2R}\left(1 - \frac{1}{D}\right)$$

where q is the charge on the ion, R is the radius of the ion, D is dielectric constants of the system. According to this treatment, the energy of electrostatic interaction or the logarithm of protonation constant (log K) should vary linearly as a function of the reciprocal of the dielectric constant ($1/D$) of the medium. Such linear variation of the protonation constants of adrenaline (Figure 2) in ethanol-water mixture shows the dominance of electrostatic interactions.

Water is substituted by ethanol which has a lower dielectric constant. Thus, the electrostatic force of attraction between ions of opposite charge is reduced. Adding ethanol decreases the dielectric constant of solution (Lide, 2004), resulting in a greater attraction force and hence larger formation and protonation and formation constants. In this study, we evaluate stability constants for Al(III) binding to adrenaline and the effect of solvent systems on protonation and complexation. Solvent effects on acid-base phenomena in amphiprotic media of intermediate and high dielectric constant (such as methanol and ethanol) are often successfully interpreted in terms of changes in the dielectric constant (electrostatic effects) and changes in the basicity (non-electrostatic effects). Accordingly, a lowering of the dielectric constant due to the addition of ethanol may have little effect on the basicity of the compound under investigation. Water is substituted by ethanol which has a lower dielectric constant. Thus, the electrostatic force of attraction between ions of opposite charge is reduced. Adding ethanol decreases the dielectric constant of solution, resulting in a greater attraction force and hence larger formation and protonation and formation constants (see Figure 2 and Table 3).

Composition of the complex of adrenaline-iron(III) and conditional stability constant

The stoichiometric ratio of adrenaline to iron(III) chloride in the complex were determined by the Job method of equimolar solutions (Vosburgh and Copper, 1941) (Figure 3). The curves display the mole ratio method (Yoe and Jones, 1944) at constant adrenaline concentration (1.0×10^{-3} M) and 40–1600 µl iron(III) chloride 1.25×10^{-3} M. The highest absorbance value at λ=480 nm was measured at pH 6. Thus, pH=6 is selected as the most suitable pH value for complex formation.

A sharp band was observed at the mole ratio adrenaline/iron(III)=2:1. The obtained results by this method confirmed the mole ratio of adrenaline.

The complex formation constant, K_f, was evaluated with absorbance-mole ratio data; the nonlinear least-squares curve-fitting program KINFIT was used. The program is based on the iterative adjustment of calculated to observed absorbance values by using either the Wentworth matrix technique (Wentworth, 1965) or the Powell procedure (Powell, 1964). Adjustable parameters are K_f and ε, where ε is the molar absorption coefficient

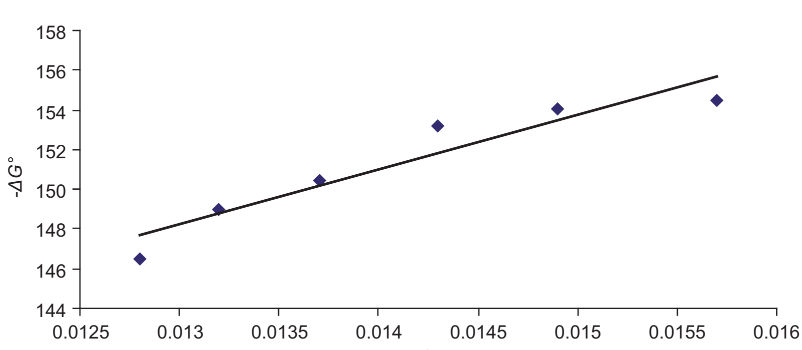

Figure 2 -$\Delta G°$ vs. $1/D$ for (x) water +(1 - x) ethanol at 25°C.

x (molar fraction)	log $K^{H}_{Al(HL)^{2+}}$	log $K_{Al(HL)^{2+}}$	-ΔG°, kJ.mol	ΔS°, J/mol.K
t=15°C				
1	4.12	25.95	142.911	496.15
0.95	4.30	26.17	144.123	500.42
0.9	4.59	26.50	145.940	506.71
0.85	5.15	27.04	148.914	517.02
0.8	5.20	27.20	149.795	520.08
0.75	5.23	27.26	150.126	521.23
				t=20°C
1	4.05	25.79	144.496	493.10
0.95	4.42	26.16	146.569	500.23
0.9	4.57	26.42	148.025	505.18
0.85	4.98	26.95	150.995	515.30
0.8	5.19	27.13	152.003	518.74
0.75	5.22	27.20	152.396	520.09
				t=25°C
1	4.04	25.70	146.448	491.37
0.95	4.43	26.15	149.013	500.03
0.9	4.63	26.40	150.437	504.80
0.85	5.13	26.88	153.172	513.96
0.8	5.18	27.03	154.027	516.83
0.75	5.20	27.11	154.483	518.36

Table 3 Average values of $K_{Al(HL)^{2+}}$ and $K^{H}_{Al(HL)^{2+}}$, $\Delta G°$ and $\Delta S°$ of Al(III) with adrenaline with standard deviations (0.01), in (x) water +(1 - x) ethanol at different temperature and I=0.2 mol.dm^{-3}.

of the complex. A computer sample fitting of the absorbance-mole ratio data for Fe^{3+} ion and adrenaline at 25°C is shown in Figure 4. The resulting K_f and thermodynamic parameters of the complex at different temperatures are listed in Table 4.

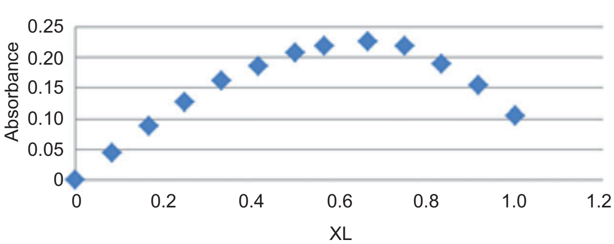

Figure 3 Continuous variation graph of adrenaline-iron(III) (1×10^{-3} M).

Temperature (°C)	log K	-ΔG°, kJ.mol	ΔS°, J/mol.K
15	7.210	39.652	591.00
20	7.365	41.265	575.42
25	7.490	42.681	561.01

Table 4 Formation constant values and thermodynamic parameters for adrenaline-iron(III) at different temperatures.

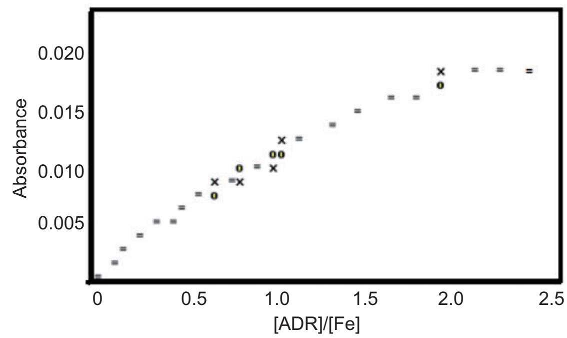

Figure 4 Computer fits of the plots of absorbance vs. [ADR]/[Fe] mole ratios at 25°C. (×) experimental point, (o) calculated point, (=) experimental and calculated points are the same within the resolution of the plots.

Conclusion

Complex formation leads to changes in numerous physical chemical properties of solutions. In principle, the measurement of any parameter which varies in response to complex formation provides a possibility for calculation of the compositions and formation constants of the complexes. One of the most frequent methods of carrying out photometric measurements is to record the spectra as a function of pH. We evaluate the stability constants and thermodynamic parameters for Al^{3+} binding to adrenaline in cosolvent systems of ethanol and water using a combination of potentiometric and spectrophotometric methods. The stoichiometries and stability constants of the complexes formed in the manganese(II), cobalt(II), nickel(II), copper(II), and zinc(II)-L-adrenaline and noradrenaline systems were determined pH-metrically at 25°C and 0.2 mol.dm^{-3} ionic strength (Kiss and Gergely, 1979b). In this study, the protonation constants of adrenaline and noradrenaline were determined. Comparison of the deprotonation constants determined in the present work and by others (Kiss and Gergely, 1981; Wolfgang et al., 1993; Coinceanainn and Hynes, 2001) reveals good agreement.

Nowadays different programs such as the KINFIT and BEST programs have been used for evaluating the stability constant of complexes or dissociation constants of ligands, using spectrophotometric or potentiometric data. In the present work, the authors decided to investigate the effect of the ligand structure on the stability constants of the complexation of the Fe(III) ion. Adrenaline contain potential metal bonding sites and will bind preferentially to hard metals such as Al(III) and Fe(III) (ionic radius 0.50 and 0.64, respectively). In hard_/soft_/acid_/base theory, the hardness of a metal ion correlates with the metal ion charge/radius ratio, so the metal ions become softer as they become larger. The Al(III) and Fe(III) ions are classified as hard and show a preference for hard oxygen donors such as those that comprise the adrenaline binding site. Stability constant depends upon the size of cations. It could be seen from Table 3 that reduction in the value of stability constant of Al(III) complexes is due to its smaller cationic size as compared with cationic size of Fe(III).

Experimental section

Reagents

Adrenaline, $AlCl_3$, $FeCl_3$, ethanol, sodium acetate, acetic acid, and sodium chloride were obtained from E. Merck (White House Station, NJ, USA) and were used without further purification.

Measurements

In method A, all measurements were carried out at 15, 20, 25±0.1°C and ionic strength 0.2 M controlled with sodium chloride. Ligand concentrations were 0.6 mmol.dm^{-3} and Al^{3+} concentration 0.2, 0.3, 0.6 mmol.dm^{-3} with ligand to Al^{3+} molar ratios of 3:1, 2:1, 1:1. The pH of solutions were controlled with acetate buffers.

In method B, for determination of the thermodynamic parameters, the solutions of adrenaline and $FeCl_3$ were prepared separately. Then, on the basis of the mole ratio method, various solutions of complex with different CM/CL mole ratios were prepared by addition of different amounts of $FeCl_3$ solutions to the fixed amounts of adrenaline solution. The absorbance of the solutions was measured in three temperatures.

A Horiba (Minami-Ku Kyoto, Japan) D-14 pH meter was employed for pH measurements. The hydrogen ion concentration was measured using an Ingold UO3234 glass electrode and an Ingold (MA, USA) UO3236 calomel electrode. It is essential that the system be calibrated routinely for various solvent mixtures of known hydrogen-ion concentration (Bates, 1973; Wu et al., 1988; Beck and Nagipal, 1990; Sadava and Pak, 1993). Spectrophotometric measurements were performed on a UV-VIS Shimadzu 2101 spectrophotometer (MD, USA) with a 486 SX/25D computer using thermostated matched 10-mm quartz cells.

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