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Potentiometric Studies on the Complexes of Tetracycline (*TC*) and Oxytetracyclin (*OTC*) with Some Metal Ions

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Summary. The interaction of Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Zn(II), Cd(II), Hg(II), Pb(II), Al(III), and UO_2(II) ions with tetracycline (*TC*) were studied by potentiometric *pH* titrations. The formation constants of the different binary complexes formed in such systems have been determined at 25 ± 0.1°C and $\mu = 0.1\,\mathrm{mol\,l^{-1}}$ ($NaNO_3$). Potentiometric *pH* equilibrium measurements have been made under the same conditions for the interaction of oxytetracycline (OTC) and Cu(II), Cd(II), Pb(II), and UO_2(II). The formation of (1 : 1) binary complexes are inferred from the potentiometric *pH* titration curves. The protonation constants of *TC* and *OTC* were also determined under the same conditions and refined (ESAB2M computer program). The transition metal stability constants are consistent with the Irving-Williams series.

Keywords. Complexes; Tetracycline; Oxytetracycline; Potentiometric studies.

Potentiometrische Untersuchungen der Komplexe von Tetracyclin (*TC*) and Oxytetracyclin (*OTC*) mit einigen Metall-Ionen

Zusammenfassung. Die Wechselwirkungen von Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Zn(II), Cd(II), Hg(II), Pb(II), Al(III) und UO_2(II) Ionen mit Tetracyclin (*TC*) wurden mittels potentiometrischer *pH*-Titrationen untersucht. Die Komplexbildungskonstanten wurden bei 25 ± 0.1 °C und $\mu = 0.1$ mol/l ($NaNO_3$) bestimmt. Unter den gleichen Bedingungen wurden die Komplexierung von Cu(II), Cd(II), Pb(II) und UO_2(II) mit Oxytetracyclin (*OTC*) mittel potentiometrischer *pH* Gleichgewichtsmessungen untersucht. Aus den potentiometrischen *pH*-Titrationskurven ergab sich die Bildung von (1 : 1) binären Komplexen. Die Protonierungskonstanten von *TC* und *OTC* wurden ebenfalls bestimmt und rechnerisch verfeinert (ESAB2M Computer Programm). Die Stabilitätskonstanten für die Übergangsmetalle stimmen mit der Irving-Williams Reihe überein.

Introduction

Among the most important broad-spectrum antibiotics are the members of the tetracycline family. The phenomenon of complex formation between tetracycline analogues and various cations is well documented [1–13]. Metal ion complex formations are among the prominent interactions in nature [15–17]. Tetracyclines form strong coordination complexes with metal ions [5]. The antibacterial activity is approximately the same [18] but its mechanism has not been definitely established, though it is believed to be associated with the formation of mixed complexes of antibiotic, ribosomes and a metal ion [19]. This prompted us to study the interacation of tetracycline (*TC*) and oxytetracycline (*OTC*) with Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Zn(II), Cd(II), Hg(II), Pb(II), Al(III), and UO_2(II) ions for an improved understanding of the driving forces leading to complex formation.

Experimental

pH measurements were carried out on a Corning 601A precision research ionalyzer digital *pH* meter. During the titrations, oxygen-free nitrogen was bubbled through the solution. The electrode system was calibrated in terms of hydrogen-ion concentrations instead of activities. Thus, all constants determined in this work are concentration constants. The accuracy of the *pH*-meter was checked making use of buffers with *pH* values of 4.008 (0.05 *M* potassium hydrogen phthalate) and 9.180 (0.01 *M* borax) at 25°C. The solutions of Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Zn(II), Cd(II), Hg(II), Pb(II), Al(III), and UO_2(II) (AR, BDH) were prepared and standardized by conventional methods. Tetracycline (*TC*) and oxytetracycline (*OTC*) were obtained as the hydrochlorides and used without purification as fresh solutions in bidistilled water.

In the binary systems solution the following sets of solutions have been titrated potentiometrically against standard carbonate-free sodium hydroxide (0.1 mol dm^{-3} solution):

(a) 0.01 mol dm^{-3} HCl;
(b) (a) + 0.01 mol dm^{-3} *TC* hydrochloride;
(c) (b) + $2.5 \cdot 10^{-3}$ mol dm^{-3} metal ion solution;
(d) 0.01 mol dm^{-3} $HClO_4$;
(e) (d) + 0.01 mol dm^{-3} OTC;
(f) (e) + $2.5 \cdot 10^{-3}$ mol dm^{-3} metal ion solution.

The total volume was adjusted to 50 cm^3 by adding double-distilled water in each case. The titration were performed at 25°C and $\mu = 0.1$ mol dm^{-3} ($NaNO_3$).

Results and Discussion

Representative titration curves are shown in Fig. 1 and Fig. 2. In tetracycline or oxytetracycline NaOH titration, the ligand and proton mass balanced equations can be solved to obtain an explicit function of the titrant volume and of the average number of protons bound to the ligand [20–21]. By minimizing the error squares sum

$$Uv = \sum_i W_i (V_i - V_{calc,\,i})^2 \qquad (1)$$

where V = titrant volume, w = weight, it is possible to refine all parameters involved in the acid-base titration [22], i.e., analytical concentrations, E^o, function potential and protonation constants. The weight is calculated by

$$\frac{1}{w_i} = S_i^2 = S_v^2 + (\delta V_i / \delta E_i)^2 S_E^2 . \qquad (2)$$

By minimizing the error squares sum

$$U_p^- = \sum_i w_i (P_i^- - P_{calc,\,i}^-)^2$$

where E = e.m.f., S^2 = estimated variance, P = the average number of protons bound to the ligand, it is possible to refine the protonation constants. The weight is calculated by:

$$\frac{1}{w_i} = S_i^2 (\delta P_i/\delta V_i)^2 S_v^2 + (\delta P_i/\delta E_i)^2 S_E^2. \tag{3}$$

Refinement of proton ligand association constants of tetracycline and oxytetracycline were carried out using the computer program ESAB2M [22]. The values of log K_{1H}, log $K_2{}^H$ and log $K_3{}^H$ (the first second and third proton association constants) of *TC* and *OTC*, as shown in Tables 1 and 2, agree quite well with those previously reported [2, 4].

The titration curves of the metal-ligand solutions as shown in Figs. 1 and 2 are well separated from the ligand solution. Thus, replacement of H^+ ions is due to complexation. The dotted portion of each curve represents the *pH* range in which precipitation was observed. From these titration curves, the values of *n* and *pL* have been calculated using the IBM PC AT computer. The corresponding values of stability constants have been calculated using the weighted least squares method of Sullivan et al. [23]. The weighted least squares treatment determines the set of β_n values which makes the function:

$$U\left[U = \sum_{n=0}^{N} (Y - X - nx)\,\beta_n X^x\right] \tag{4}$$

nearest to zero, my minimizing

$$S\left[S = \sum_{i=1}^{I} U^2(X_i Y_i Z_i)\right] \tag{5}$$

with respect to the variation in β_n. We report the S_{min} values for different metal complexes. S_{min} has the same statistical distribution as X^2 with *K* degrees of freedom

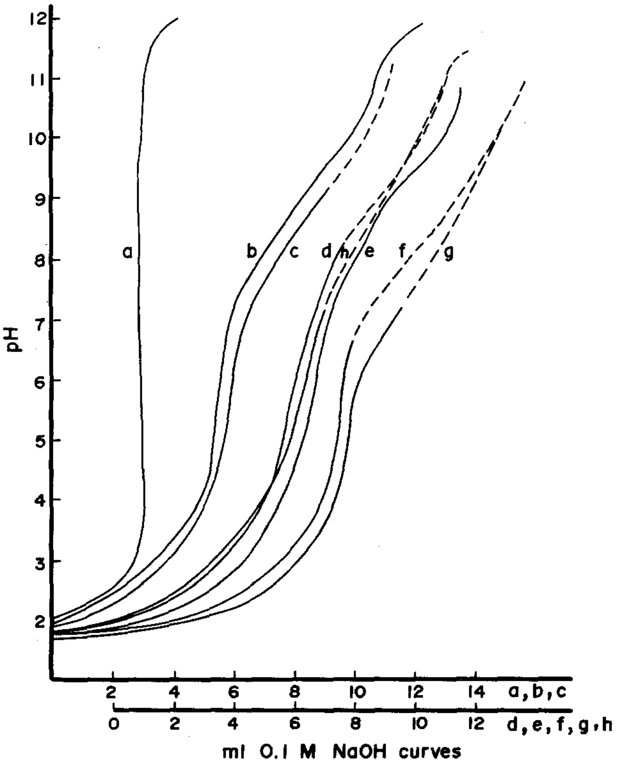

Fig. 1. Potentiometric titration curves of *TC* with metal ions: *a.* 0.01 *M* HCl+0.09 *M* NaNO$_3$, *b* a+0.1 *M TC*, *c* b+2.5 m*M* Zn(II), *d* b+2.5 m*M* Mn(II), *e* b+2.5 m*M* Ni(II), *f* b+2.5 m*M* Hg(II), *g* b+2.5 m*M* Cd(II), *h* b+2.5 m*M* Pb(II)

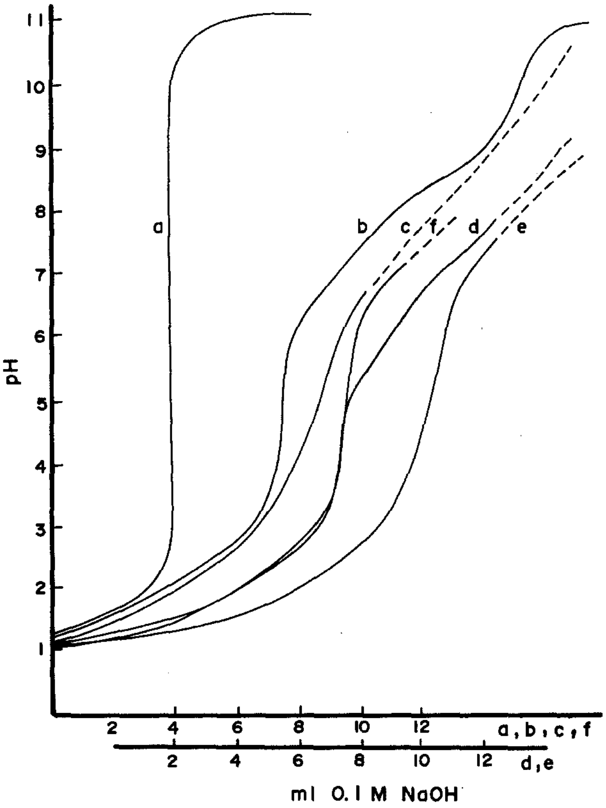

Fig. 2. Potentiometric titration curves of *OTC* with metal ions: *a* 0.01 *M* $HClO_4$, *b* a + 0.01 *M* *OTC*, *c* b + 2.5 m*M* Pb(II), *d* b + 2.5 m*M* Cd(II), *e* b + 2.5 m*M* Cu(II), *f* b + 0.1 m*M* UO_2(II)

and with weights defined in accordance with Rydberg et al. [24]. S_{min} can be equated to X^2. The stability constants thus calculated are given in Tables 1 and 2. For all the metal ions studied it was only possible to obtain the formation constants of the first complex since the formation of the second one is disturbed by hydrolysis and precipitation of the metal ion. Therefore, the experimental data including measurements in the *pH* range where hydrolysis occurs were not taken into account, since data in this *pH* range would not be useful in the numerical calculations. Moreover, these data cannot be considered to be at equilibrium, since the *pH* readings at this stage showed an unsteady drift.

Tetracycline and oxytetracycline hydrochlorides $LH_3{}^+Cl^-$ has three sites and the protons may be dissociated from these sites in proportions which vary with the degree of neutralization. These sites are shown in Fig. 3 as the amide system (A), the phenolic B-diketone moieties (B) and (B′), and the dimethyl-ammonium cation (C): the first dissociation constant $pK_1{}^H$ is generally attributed to site (A) [26, 27]. Stephens et al. [26] have assigned the second and the third dissociation constants, $pK_2{}^H$ and $pK_3{}^H$, to site (C) and combined (B) and (B′) system, respectively, while Lesson et al. [27] reverse these assignments in the case of protonated tetracycline, considering a complete dissociation scheme for all. Sachan [5] suggested that $pK_2{}^H$ is due to system (B). Doluisio and Martin [1] concluded that the chelation occurs through the coordination of the nitrogen atom of the dimethylamino group of C_4 and the oxygen atom of the hydroxyl group either at C_3 or at C_{12a}. On basis of the absorption spectra Conover [28] concluded that the normal site for metal binding by tetracycline is the enolized B-diketone group at C_{11} and C_{12}, i.e. group

Table 1. Formation constants of metal complexes with tetracycline at $\mu = 0.1\ \mathrm{mol\,dm^{-3}}$ ($NaNO_3$) and $t = 25\,^{\circ}\mathrm{C}$

Central ion	log K_1	log K_2	log K_3	log K_4	log K_5	Ref.
H^+	10.86 ± 0.072	8.5 ± 0.06	3.56 ± 0.05			present work
	9.57	7.82	3.35			[2]
	10.70	9.69	7.68	3.30		[14]
	8.85	8.15	7.42	4.75	3.18	[25]
Al(III)	12.5 ± 0.033	–	–	–	–	present work
S_{min}	0.118					
	7.4	6.4	5.4			[2]
Co(II)	10.4 ± 0.042	–				present work
S_{min}	0.178					
	5.4	4.4				[2]
	29.32	25.10				[25]
Fe(III)	13.4 ± 0.063	–				present work
S_{min}	0.136	–				
	9.9	8.6	6.8			[2]
	35.7	19.0				[25]
Mn(II)	3.9 ± 0.032	–				present work
S_{min}	0.123					
	4.4					[2]
	27.7	23.42				[25]
Ni(II)	10.5 ± 0.043	–				present work
S_{min}	0.117					
	6.0	5.0				[2]
	28.6	22.42				[25]
Zn(II)	4.3 ± 0.022	–				present work
S_{min}	0.158					
	4.9	–				[2]
	28.35	24.65				[25]
Hg(II)	6.5 ± 0.053	–				present work
S_{min}	0.68					
Cr(III)	9.0 ± 0.062					present work
S_{min}	0.149					
	19.60	17.40				[25]
Cd(II)	7.4 ± 0.031	–				present work
S_{min}	0.92					
Pb(II)	8.3 ± 0.042	–				present work
S_{min}	0.117					
UO_2(II)	4.6 ± 0.033	–				present work
S_{min}	0.136					

Table 2. Formation constants of metal complexes with oxytetracycline

Central ion	log K_1	log K_2	log K_3	log K_4	log K_5	Ref.
H^+	9.48 ± 0.052	7.55 ± 0.061	2.78 ± 0.022	–	–	present work
	9.11	7.26	3.10			[2]
	8.82	8.05	7.31	5.80	3.15	[25]
UO_2(II)	4.97 ± 0.053	–	–	–	–	present work
S_{min}	0.136					
Cu(II)	6.98 ± 0.062					present work
S_{min}	0.162					
	7.2	5.0				[2]
	30.30	25.06				[25]
Cd(II)	7.83 ± 0.045					present work
S_{min}	0.149					
Pb(II)	9.82 ± 0.073					present work
S_{min}	0.126					

(B′) in Fig. 3. According to Baker and Brown [29] on the basis of reflectance spectra, the dimethylamino group does not take part in the coordination, and the chelation is through oxygen atoms, probably in system (A). Sachan [5] concluded that the coordination sites of *TC* and *OTC* are the oxygen atoms of system (B).

Thus, there is lack of agreement as to the assignment of the site of coordination. The following considerations may be suggested:

a) Albert [30] pointed out that only the functional group having a dissociation constant of approximately 7.0 undergoes ionization when chelation takes place.

b) The presence of an OH group at C_3 suggests that the complexation of metal ions with *TC* and *OTC* should be independent of $pK_1{}^H$, and consequently the complexing species should not be LH_2 [6].

c) Covalent forces, rather than electrovalent forces, are dominating in the phenol-β-diketone system and in the dimethyl ammonium group between O and H atoms at C_{10} and between N and H atoms at C_4, respectively [4]. From these we may assume that the complexation of Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Zn(II), Cd(II), Hg(II), Pb(II), Al(III), and UO_2(II) with *TC* and Cd(II), Pb(II), Cu(II), UO_2(II) with *OTC* takes place through the $pK_2{}^H$ value of the antibiotic, and that the complexing species were LH^- for these systems. Considering that

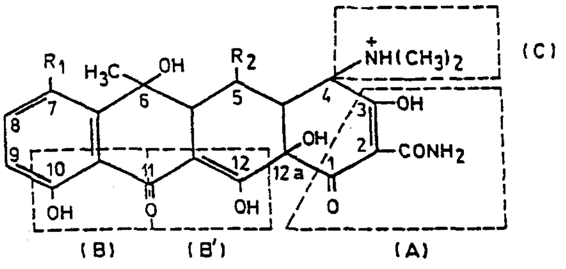

Fig. 3. Complexing sites of *TC* ($R_1 = R_2 = H$) and *OTC* ($R_1 = H$, $R_2 = OH$)

metal complexation may be involved in the antibacterial activity [31–33] and that the phenolic group in tetracycline [34] is responsible for part of its antibacterical activity, this may be considered to present further support for this conclusion.

The order of stability of the different binary complexes formed between *TC* and transition metal ions investigated in this study is in the expected Irving-Williams order: Mn(II) < CO(II) < Ni(II) < Zn(II).

Inspection of the formation constant values (Table 1) in terms of the nature of metal ion reveals that the stability of the complex species (*M* – *TC*) increases in the following order: Fe(III) (r = 0.63 Å) > Al(III) (0.053 Å) > Cr(III) (0.76 Å) > Pb(II) (1.33 Å) > Cd(II) (0.92 Å) > Hg(II) (0.83 Å). It is evident that the stability of the 1 : 1 complex species does not follow the increase in the net charge carried by the metal ions. This suggests that the nature of forces in *TC* metal complexes is almost exclusively covalent.

Looking at our results it can be seen that the stoichiometry and the formation constants of only Mn(II) and Zn(II) complexes with *TC* are in a fairly good agreement with those reported by Albert [2]. Comparison of the formation constants with literature values [2] for the complexes of Hg(II), Cd(II), Pb(II), Cr(III) or UO_2(II) with *TC* and Cd(II), Pb(II) or UO_2(II) with *OTC* is difficult due to the lack of data concerning these systems. Furthermore, there are differences in both stoichiometry and formation constants of the other metal tetracycline systems viz; Al(III), Co(II), Fe(III), and Ni(II). This disagreement may be mainly due to the different ionic strengths used. Also, the disagreement between our results and those of Ref. [25] is quite clear. The very high stability constants reported in this work cannot be logically explained.

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