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Equilibrium studies of binary and ternary complexes of oxytetracycline and amino acid or DNA units

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Abstract. The acid-dissociation constants of oxytetracycline (OTC) and the formation constants of the copper(II) complexes were determined at $t = 25°C$ and $\mu = 0{\cdot}1\ \text{mol dm}^{-3}$ ($NaNO_3$) in dioxane–water mixtures. The mixed-ligand complexes of copper(II) with OTC as primary ligand and amino acids or DNA constituents as secondary ligands were investigated in 50% v/v dioxane–water mixture. The formation constants of the complexes formed in solutions and their concentration distributions as a function of *p*H were evaluated. The relative stabilities of the ternary and corresponding binary complexes were also studied.

Keywords. Oxytetracycline; acid-dissociation constants; binary and ternary complexes.

1. Introduction

Tetracyclines are a family of broad-spectrum antibiotics of known prophylactic and therapeutic values. They contain electron-donor groups likely to bind metal ions occurring *in vivo* (Bojczuk *et al* 1993). So, tetracycline antibiotics behave as relatively efficient chelating agents (Albert 1953). The antibacterial activity is believed to arise from the formation of mixed complexes of the antibiotic and bacterial nucleic acid. Complexes formed between copper and tetracyclines may indeed be stable enough to mobilize a significant part of the low-molecular-weight fraction of this metal ion and, hence, facilitate its tissue penetration or its excretion (Williams *et al* 1982; Berthon *et al* 1984). The possible interference of the antibiotic with copper metabolism appears to be of special interest since plasma concentrations of this metal are known to fluctuate during the evolution of inflammatory diseases (Sorenson *et al* 1984).

Although metal complexes of tetracyclines have been extensively investigated, the mixed-ligand complexes of oxytetracycline have not been investigated to date. In connection with our previous studies of the binary and ternary complexes of cephradine antibiotics (Shoukry *et al* 1995), the present investigation reports potentiometric studies of the binary and ternary complexes of copper(II) with OTC as primary ligand and amino acid or DNA units as secondary ligands. The structural formulae of OTC and DNA units are given in figure 1.

2. Experimental

2.1 *Materials*

Oxytetracycline hydrochloride (OTC) was kindly supplied by El-Nile Chem. Co., Egypt. On account of the well-documented instability of tetracyclines in aqueous

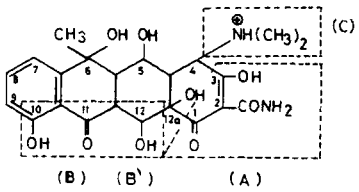

Figure 1. Structural formulae of oxytetracycline.

media (Mitscher *et al* 1968; Dockter and Magnuson 1974), OTC solutions were freshly prepared prior to use and systematically analysed for acid contents, by means of the appropriate potentiometric titrations (Rossotti and Rossotti 1965). The solutions were stored in the dark under an atmosphere of purified nitrogen. The amino acid and DNA units used were from Sigma Chem. Co. Adenine solution was prepared by dissolving it in aqueous equimolar nitric acid solution. Copper nitrate was provided by BDH. The copper content of the solution was estimated complexometrically (Welcher 1965). Sodium hydroxide stock solutions were prepared by diluting the contents of BDH concentrated volumetric solution vials. These solutions were systematically checked by titration against potassium hydrogen phthalate. All solutions were prepared in freshly boiled doubly deionised water.

2.2 *Procedure*

Potentiometric measurements were carried out as described previously (Bates 1973). Protonation constants of the ligands were determined by titrating 40 ml of the ligand solutions ($2{\cdot}5 \times 10^{-3}$ M) and $NaNO_3$ (0·1 M) at 25°C. The stability constants of the binary complexes were determined under the same conditions as the protonation constants but $NaNO_3$ was partly replaced by $Cu(NO_3)_2$ in the ratio $[Cu^{2+}]:[OTC] = 1:1$ for the Cu-OTC binary complex and the ratio 1:2 for the Cu-D binary complex (where D = amino acid or DNA units). The conditions for titrations of the ternary Cu-OTC-D complexes were the same as those for the binary ones, but the solutions contained equivalent amounts of Cu^{2+}, OTC and D. The data obtained, through the *p*H range corresponding to the complete formation of the Cu-OTC complex, were used for the determination of the stability constants $K^{Cu(OTC)}_{Cu(OTC)D}$ for the ternary complexes. The calculations were restricted to data obtained at *p*H values below 9 or before precipitation, in order to avoid complications due to the hydrolysis of the complex species at higher *p*H values. Direct *p*H-meter readings were used in the calculations; no corrections were applied for the change in solvent from water to mixed solvent (Sigel *et al* 1985). The calculations were made with the aid of the MINIQUAD-75 computer program (Gans *et al* 1976) on an IBM-486 computer.

3. Results and discussion

3.1 *Protonation equilibria*

The acid-dissociation constants of the oxytetracycline, table 1, correspond to the dissociation of three protons, from site (A), combined (B) and (B′) system and site (C), which are pK^H_1, pK^H_2 and pK^H_3 respectively. The acid dissociation of site (A), i.e. the

Table 1. Effect of dioxane on the dissociation constant of oxytetracycline and formation constants of copper(II)–oxytetracycline complex.

System	% Dioxane (v/v)	pK_1^{Ha}	pK_2^H	pK_3^H	*p*H range	S^b ($\times 10^{-8}$)
Oxytetra-cycline	00·0	3·61 (0·02)	7·68 (0·02)	9·34 (0·01)	3·31 – 10·00	3·1
	25·0	3·81 (0·02)	7·78 (0·01)	9·44 (0·01)	3·40 – 10·50	6·8
	37·5	4·06 (0·03)	7·88 (0·02)	9·51 (0·01)	3·54 – 10·72	8·3
	50·0	4·20 (0·02)	7·83 (0·01)	9·32 (0·07)	3·62 – 10·16	3·2
	62·5	4·40 (0·02)	7·80 (0·01)	9·34 (0·01)	3·69 – 10·71	6·3
	75·0	4·48 (0·02)	7·82 (0·01)	9·36 (0·01)	3·61 – 10·69	3·7

System	% Dioxane (v/v)	$\log\beta_{CuL}$	$\log\beta_{CuLH}$	*p*H range	S^b ($\times 10^{-7}$)
Cu–Oxyte-tracycline	0·00	12·11 (0·03)	18·56 (0·02)	2·74 – 7·23	1·3
	25·0	12·25 (0·03)	18·67 (0·02)	2·71 – 7·11	1·5
	37·5	12·46 (0·07)	19·09 (0·02)	2·71 – 6·96	1·6
	50·0	12·65 (0·04)	18·95 (0·02)	2·69 – 6·74	2·3
	62·5	13·59 (0·04)	19·66 (0·03)	2·64 – 6·56	3·2
	75·0	14·00 (0·08)	20·07 (0·05)	2·49 – 6·38	3·5

[a]Standard deviations are given in parentheses, [b]Sum of square of residuals

amide system, is due to the O(3)-H group adjacent to the amide group. The pK_a of the third phenolic OH group was not determined owing to its value being too high to be determined by potentiometric techniques.

3.2 *Complex formation equilibria*

The binary Cu-OTC complex formation equilibria were investigated in dioxane–water solutions of different compositions. The potentiometric data could be fitted considering the formation of Cu(OTC) and Cu(OTC)H complexes and their formation constants are given in table 1. Preliminary information on the bonding modes of copper(II) complex could be examined through the study of the corresponding stability constants in table 1. Assuming that the ligand-proton interactions remain unaffected in the presence of copper, stability of the metal–ligand bond formed in the protonated complex species [$\beta_{Cu(HL)}$] in 50% dioxane-water solutions, can be calculated using the equation,

$$\log\beta_{Cu(HL)} = \log\beta_{CuHL} - \log\beta_{HL}, \quad (1)$$

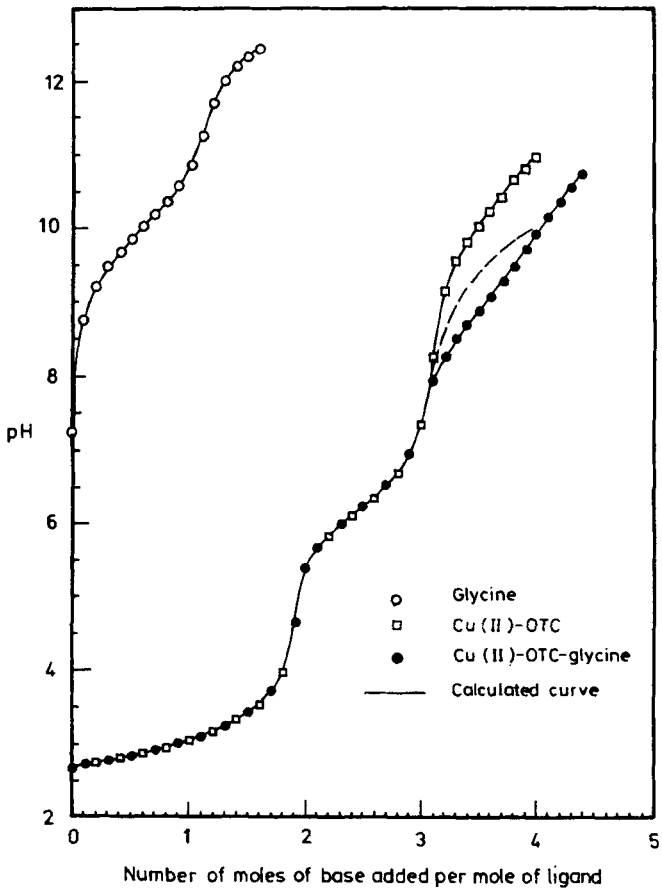

Figure 2. Potentiometric titration curves of Cu(II)-OTC-glycine system.

where (HL) represents the monoprotonated form of oxytetracycline, β_{HL} and β_{CuHL} express step-protonation increment and parent overall formation constant respectively. The calculated $\log\beta_{Cu(HL)}$ value (9·63) is much smaller than the $\log\beta_{CuL}$ value (12·65), indicating important changes in the copper coordination mode in the *p*H range investigated. Chelation in the CuHL complex is suggested to take place in the O(10)-O(12) system. In the CuL complex, the dianionic form of oxytetracycline binds to copper(II) through the N(4) atom. This is in agreement with the data of the copper(II) complex of 6-desoxy-6-demethyltetracycline (Bojczuk *et al* 1993), which has a structure similar to that of oxytetracycline. It is to be mentioned that the coordination ability of the $CONH_2$ is assumed to be low since the electron-withdrawing carbonyl group decreases the electron density on the $-NH_2$ group thereby making its coordination potential very low.

Potentiometric titration curves of the Cu-OTC system obtained in presence and absence of glycine, taken as a representative, are shown in figure 2. The curves are nearly coincident in the region $0 \leqslant a \leqslant 3$ (a = number of moles of base added per mole of ligand), in which region, only 1:1 Cu(II)-OTC complex is formed, probably due to its high stability compared to that of Cu(II)–amino acid or DNA units (D), (table 2). The formation of a ternary complex beyond the above region, can be visualized by

Table 2. Formation constants of binary (A) and ternary (B) complexes involving Cu, oxytetracycline and amino acid or DNA units in 50% dioxane.

(A) Binary complexes

System	*l*	*p*	*q*[a]	logβ[b]	*S*[c]
Cu-Glycine	0	1	1	9·82 (0·00)	$4{\cdot}2 \times 10^{-8}$
	1	1	0	8·64 (0·03)	$2{\cdot}1 \times 10^{-7}$
	1	2	0	16·60 (0·03)	
Cu-Alanine	0	1	1	9·78 (0·01)	$1{\cdot}6 \times 10^{-7}$
	1	1	0	8·52 (0·03)	$3{\cdot}1 \times 10^{-7}$
	1	2	0	16·36 (0·03)	
Cu-Proline	0	1	1	10.71 (0·01)	$1{\cdot}8 \times 10^{-7}$
	1	1	0	9·57 (0·02)	$3{\cdot}3 \times 10^{-7}$
	1	2	0	17·90 (0·03)	
Cu-Valine	0	1	1	9·66 (0·01)	$6{\cdot}5 \times 10^{-7}$
	1	1	0	8·30 (0·05)	$2{\cdot}2 \times 10^{-7}$
	1	2	0	16·59 (0·02)	
Cu-Hydroxyproline	0	1	1	9·85 (0·01)	$3{\cdot}1 \times 10^{-7}$
	1	1	0	9·08 (0·02)	$1{\cdot}9 \times 10^{-7}$
	1	2	0	17·45 (0·02)	
Cu-β-Phenylalanine	0	1	1	9·28 (0·01)	$8{\cdot}8 \times 10^{-8}$
	1	1	0	7·91 (0·05)	$2{\cdot}8 \times 10^{-7}$
	1	2	0	16·12 (0·02)	
Cu-S-Methylcysteine	0	1	1	9·08 (0·01)	$7{\cdot}2 \times 10^{-8}$
	1	1	0	7·64 (0·08)	$3{\cdot}9 \times 10^{-8}$
	1	2	0	16·15 (0·01)	
Cu-iso-Leucine	0	1	1	9·88 (0·02)	$2{\cdot}4 \times 10^{-7}$
	1	1	0	8·32 (0·05)	$3{\cdot}9 \times 10^{-7}$
	1	2	0	16·80 (0·02)	

(*Continued*)

Table 2. (*Continued*)

System	*l*	*p*	*q*[a]	logβ[b]	*S*[c]
Cu-2-Amino-*n*-butyric acid	0	1	1	9·81 (0·01)	$2{\cdot}1 \times 10^{-7}$
	1	1	0	8·32 (0·04)	$3{\cdot}6 \times 10^{-7}$
	1	2	0	16·45 (0·02)	
Cu-Threonine	0	1	1	9·26 (0·01)	$1{\cdot}9 \times 10^{-8}$
	1	1	0	7·96 (0·05)	$2{\cdot}6 \times 10^{-7}$
	1	2	0	16·14 (0·02)	
Cu-Methionine	0	1	1	9·39 (0·01)	$9{\cdot}9 \times 10^{-8}$
	1	1	0	8·32 (0·04)	$3{\cdot}5 \times 10^{-7}$
	1	2	0	16·37 (0·02)	
Cu-Serine	0	1	1	9·34 (0·00)	$4{\cdot}3 \times 10^{-8}$
	1	1	0	8·40 (0·03)	$5{\cdot}3 \times 10^{-7}$
	1	2	0	16·30 (0·02)	
Cu-Histidine	0	1	1	9·66 (0·01)	$2{\cdot}5 \times 10^{-7}$
	0	1	2	15·54 (0·02)	
	1	1	0	11·31 (0·02)	$5{\cdot}5 \times 10^{-7}$
	1	2	0	19·72 (0·06)	
Cu-Histamine	0	1	1	9·40 (0·01)	$1{\cdot}4 \times 10^{-7}$
	0	1	2	14·95 (0·01)	
	1	1	0	9·48 (0·01)	$1{\cdot}8 \times 10^{-8}$
	1	2	0	15·60 (0·01)	
	1	1	1	12·67 (0·01)	
Cu-Ornithine	0	1	1	10·76 (0·01)	$3{\cdot}4 \times 10^{-7}$
	0	1	2	19·58 (0·02)	
	1	1	0	13·62 (0·04)	$1{\cdot}6 \times 10^{-7}$
	1	2	0	17·39 (0·05)	
	1	1	1	18·70 (0·03)	

(*Continued*)

Table 2. (*Continued*)

System	*l*	*p*	*q*[a]	logβ[b]	*S*[c]
Cu-Lysine	0	1	1	10·79 (0·01)	$2{\cdot}4 \times 10^{-7}$
	0	1	2	20·02 (0·01)	
	1	1	0	14·10 (0·05)	$2{\cdot}7 \times 10^{-7}$
	1	2	0	17·38 (0·07)	
	1	1	1	19·04 (0·04)	
Cu-Aspartic acid	0	1	1	10·13 (0·01)	$1{\cdot}2 \times 10^{-7}$
	0	1	2	14·82 (0·02)	
	1	1	0	9·69 (0·01)	$4{\cdot}6 \times 10^{-8}$
	1	2	0	16·56 (0·03)	
	1	1	1	13·97 (0·02)	
Cu-Inosine	0	1	1	9·16 (0·01)	$9{\cdot}4 \times 10^{-8}$
	1	1	0	6·47 (0·09)	$8{\cdot}5 \times 10^{-7}$
	1	2	0	11·74 (0·06)	
Cu-Uracil	0	1	1	10·18 (0·01)	$4{\cdot}3 \times 10^{-8}$
	1	1	0	6·21 (0·04)	$4{\cdot}2 \times 10^{-7}$
Cu-Uridine	0	1	1	10·33 (0·01)	$8{\cdot}7 \times 10^{-8}$
	1	1	0	7·02 (0·09)	$3{\cdot}7 \times 10^{-7}$

(B) Ternary complexes

System	*l*	*p*	*q*[a]	logβ[b]	*S*[c]	$\Delta \log K$	Maximun percentage	*p*H
Cu-OTC-glycine	1	1	0	3·81 (0·03)	$7{\cdot}3 \times 10^{-8}$	−4·83	29·25	10·8
Cu-OTC-alanine	1	1	0	3·67 (0·02)	$2{\cdot}4 \times 10^{-7}$	−4·85	24·40	10·8
Cu-OTC-proline	1	1	0	4·47 (0·01)	$3{\cdot}1 \times 10^{-10}$	−5·1	49·58	11·0
Cu-OTC-valine	1	1	0	3·83 (0·02)	$2{\cdot}0 \times 10^{-7}$	−4·47	30·88	11·0
Cu-OTC-hydroxy-proline	1	1	0	4·36 (0·02)	$2{\cdot}5 \times 10^{-7}$	−4·72	51·11	11·0

(*Continued*)

Table 2. (*Continued*)

System	*l*	*p*	*q*[a]	log*β*[b]	*S*[c]	Δlog*K*	Maximun percentage	*p*H
Cu-OTC-*β*-phenyl alanine	1	1	0	3·43 (0·01)	$3{\cdot}2 \times 10^{-8}$	−4·48	17·70	10·8
Cu-OTC-S-methyl cysteine	1	1	0	3·07 (0·02)	$3{\cdot}2 \times 10^{-7}$	−4·57	9·37	10·6
Cu-OTC-iso-leucine	1	1	0	4·22 (0·02)	$2{\cdot}0 \times 10^{-7}$	−4·10	45·61	11·0
Cu-OTC-2-amino-*n*-butyric acid	1	1	0	3·88 (0·02)	$3{\cdot}2 \times 10^{-7}$	−4·44	31·90	10·8
Cu-OTC-threonine	1	1	0	3·46 (0·01)	$4{\cdot}5 \times 10^{-8}$	−4·50	18·58	10·8
Cu-OTC-methionine	1	1	0	3·46 (0·01)	$3{\cdot}4 \times 10^{-8}$	−4·86	18·46	10·8
Cu-OTC-serine	1	1	0	3·49 (0·01)	$8{\cdot}9 \times 10^{-8}$	−4·91	19·15	10·6
Cu-OTC-histidine	1	1	0	3·87 (0·08)	$1{\cdot}7 \times 10^{-6}$	−7·44	31·98	10·8
Cu-OTC-histamine	1	1	0	4·25 (0·09)	$3{\cdot}3 \times 10^{-6}$	−5·23	47·37	10·8
Cu-OTC-ornithine	1	1	0	4·25 (0·03)	$1{\cdot}7 \times 10^{-7}$	−9·37	40·23	11·0
	1	1	1	9·28 (0·06)				
Cu-OTC-lysine	1	1	0	4·35 (0·03)	$2{\cdot}2 \times 10^{-7}$	−9·75	46·31	11·2
	1	1	1	9·71 (0·05)				
Cu-OTC-aspartic acid	1	1	0	4·59 (0·04)	$2{\cdot}5 \times 10^{-7}$	−5·10	58·59	11·0
Cu-OTC-inosine	1	1	0	2·60 (0·05)	$5{\cdot}3 \times 10^{-7}$	−3·87	3·64	11·0
Cu-OTC-uracil	1	1	0	3·37 (0·04)	$1{\cdot}0 \times 10^{-6}$	−2·84	14·78	11·0
Cu-OTC-uridine	1	1	0	3·59 (0·06)	$4{\cdot}1 \times 10^{-7}$	−3·43	20·33	11·0

[a]*l*, *p* and *q* are the stoichiometric coefficient corresponding to Cu(II)-OTC complex, amino acid or DNA unit and H^+ respectively; [b]Standard deviations are given in parentheses; [c]sum of squares of residuals.

comparison of the mixed ligand titration curve with the composite curve obtained by graphical addition of the secondary ligand titration data to that of the Cu(II)-OTC titration curve. Considerable deviation occurs between the mixed ligand and the resulting calculated curve indicating the formation of a ternary complex. Consequently, it may be plausibly assumed that in the presence of both ligands, OTC is first ligated to the Cu(II) ion, followed by ligation of the secondary ligand, i.e., the formation of the ternary complex may be conceived as a stepwise process, involving the equilibria,

$$\text{Cu} + \text{OTC} \rightleftharpoons \text{Cu-OTC}, \tag{2}$$

$$(\text{Cu-OTC}) + \text{D} \rightleftharpoons (\text{Cu-OTC})\text{-D}, \tag{3}$$

(charges are omitted for clarity).

The potentiometric data obtained for the ternary complexes were fitted assuming the formation of the Cu(OTC)D complex, where *D* is the deprotonated form of the secondary ligand. The corresponding stability constants are given in table 2.

3.3 *Complex formation equilibria involving DNA units*

The neutral form of inosine bears a dissociable proton at N_1. In acid medium it is protonated at N_7 with the formation of N_1H and N_7H monocations. So, inosine has two donor sites, N_1 and N_7. The complex formation in acidic medium reported that N_1 remains protonated, while the metal ion is attached to N_7 (Wang and Li 1968). The gradual change from N_7-binding to N_1-binding with increasing *p*H has been rather extensively documented by ^{1}HNMR (Maskos 1981) and EPR (Maskos 1985) spectroscopic measurements. The potentiometric data of mixed-ligand complex involving inosine showed the formation of the Cu(OTC)D species, where *D* is the anion of inosine. According to this information, the structure of the mixed ligand complex studied in the *p*H range (7–9) involves N_1 binding.

The dioxo forms (figure 1) have been established to be the predominant tautomers of uracil and uridine (Angell 1961; Katrizky and Waring 1962; Roberts *et al* 1965; Lord and Thomas 1967). For both ligands, the protonation constant values obtained show that this equilibrium is that of protonation of the [N(3)-C(4)O] grouping. The values have also been compared with those of the protonation of the analogous [N(1)-C(6)O] grouping in inosine and show that the purinic derivatives are slightly more acidic than the pyrimidinic ones. This property refers to the presence of two condensed rings in the purinic derivatives where a higher number of resonant forms will be in equilibrium. The data obtained indicate that uracil and uridine are ligating, in the deprotonated form, through N_3. Potentiometric titration data for the ternary complexes Cu-OTC-cytosine or cytidine show the absence of any significant interaction be/tween Cu-OTC complex and cytosine or cytidine along the horizontal addition procedures. This behaviour is in agreement with that found for the M-EDTA-cytosine or cytidine system (Ramalingam and Krishnamoorthy 1982).

The formation constants of binary copper(II) complexes with amino acids and DNA were previously reported in aqueous solutions (Perrin 1965, 1971). These constants are redetermined under different experimental conditions (50% dioxane) used for determining the stability constants of the ternary complexes. The relative stabilities of the ternary and binary complexes can be quantitatively expressed in a number of different ways. It has been argued that comparison can be made in terms of $\Delta \log K$ values (Martin and Prados 1974). Based on our experimental data, $\Delta \log K$ values were calculated using the relation,

$$\Delta \log K = \log K^{Cu(OTC)}_{Cu(OTC)D} - \log K^{Cu}_{Cu(D)}. \tag{4}$$

The values thus obtained are invariably negative, table 2. This means that the amino acid or DNA units form stabler complexes with the free copper(II) ion than with the Cu-OTC complex. This is expected statistically since more coordination sites are available for binding the ligand (D) with free Cu(II) ions than the Cu-OTC complex.

3.4 *Speciation distribution*

Estimation of the concentration distribution of various complex species in solution as a function of *p*H provides a useful picture of metal ion binding toward the ligands in

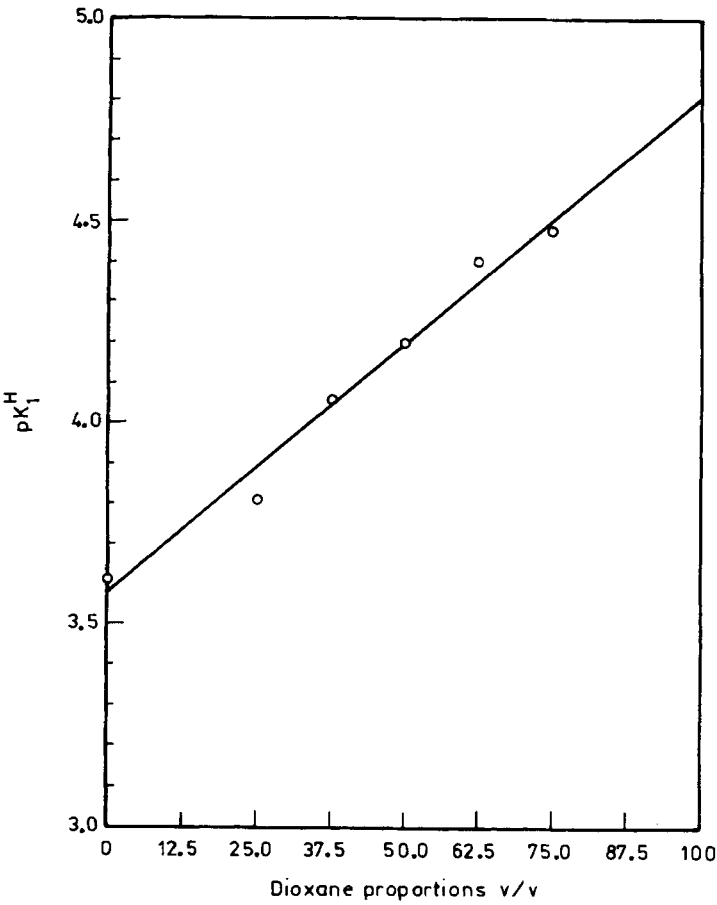

Figure 3. Effect of dioxane proportions on pK_1^H of oxytetracycline (OTC).

biological systems. All the species distributions show that the concentration of the ternary complexes increases with increasing *p*H, indicating more favourable complex formation in the *p*H range of biological systems. The values of log β (table 2) show more favourable ternary Cu(II)-OTC-amino acid complexes than those of DNA units. Thus, under physiological conditions, amino acids react more favourably with the Cu-(II)–OTC complex than DNA units.

3.5 *Effect of solvent*

Table 1 includes the protonation constants of OTC and the formation constant of the Cu(II)–OTC complex at various concentrations of dioxane-water mixture. Figure 3 represents the variation of pK_1^H values as dioxane composition in the solution mixture increases. The results obtained show a linear increase of the first dissociation constant pK_1^H with the increase of dioxane proportion in solution. This increase of pK_1^H is due to the ability of a solvent of low dielectric constant, such as dioxane, to increase the electrostatic forces between the ions which facilitate the formation of molecular species (Shoukry *et al* 1995). At low dioxane concentrations, up to 37·5% v/v, the pK_2^H and pK_3^H

values of OTC increase by increasing the dioxane content, but at higher concentrations they decrease (table 1). The decrease in pK^H values can be interpreted by the non-electrostatic forces which could include geometrical aspects, hydrogen bonding and solvent–solute interactions. This behaviour is in agreement with that proposed for oxines (Pasternack *et al* 1972). Table 1 shows different changes in the increase of the formation constant, $\log\beta_{CuL}$, of the copper(II)-OTC complex by increasing the dioxane content. At low dioxane concentrations, up to 50% v/v, the increase of $\log\beta_{CuL}$ is lower than that at higher dioxane concentrations. This behaviour may be due to the variation of the protonation ability of the O(3)H group (site A), which is one of the binding sites in the complex, as the organic solvent content increases.

4. Conclusion

The present investigation studied the formation constants of binary and ternary copper(II) complexes involving OTC, amino acids and DNA units in 50% dioxane–water solution. The conditions were similar to that in biological system and hence may have important biological implications. The favourable formation of ternary complexes of copper(II) with various ligands occurring *in vivo* shows the structural flexibility of the bioactive oxytetracycline, in addition to its ability to coordinate copper(II) through three different donor sites. In particular, bacterial nucleic acids may be involved in forming ternary complexes with the Cu-OTC complex.

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