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Equilibrium studies on tetracycline–metal ion systems[1]

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Abstract

Protonation and equilibrium constant for oxytetracycline (OTC) and doxycycline (DOX) with Zn^{2+}, Ca^{2+} and Mg^{2+} ions have been determined with Calvin-type pH-metric titrations under physiological conditions (37°C, 0.15 M NaCl ionic strength). Even though OTC and DOX are similar in structure, major differences were found in complex composition with regard to the protonation state: OTC generally formed species with less protons compared to those with DOX. Studies of parent complexes were followed by investigations of ternary complexes where ascorbic acid was used as a secondary ligand. Again, OTC showed a tendency to form complexes in which fewer protons are bound than in those with DOX. This equilibrum difference between OTC and DOX might be because DOX has a better pharmacodynamic effect relative to that of OTC.

Keywords: Equilibrium constants; Metal complexes; Potentiometry; Ternary complexes; Tetracyclines

1. Introduction

The tetracycline antibiotics that were introduced in 1948 [1] are medicines widely used against microbial infections [2,3]. Tetracyclines with a unique structure are amphoteric compounds because of the presence of both Lewis base and Lewis acid functional groups. The positions of the donor groups make tetracyclines chelate-forming ligands with numerous metal ions. This metal complex formation has been reported as being responsible for interfering with the process of bacterial growth [4]. Considering both the acid–base properties of tetracyclines and the metal ion content of the human body it is important to reveal how these ligands react with protons and different metal ions in vitro.

The first protonation and metal complex formation data for a few tetracyclines were published by Albert [5,6]; later papers reported new results [7–23]. However, in these papers many of the experimental conditions differed between papers: the ligands, the metal ions, and the methods applied were all varied by the authors. In addition, temperature and ionic strength were not consistent over the period of study.

With a view to the importance of adminsitration of the tetracyclines in medicinal practice, it was decided to perform an equilibrium study where the physiological conditions (37°C and 0.15

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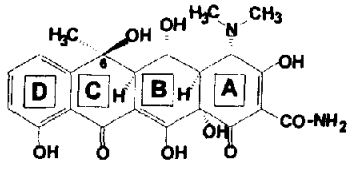

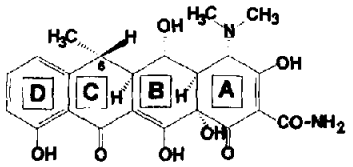

Fig. 1. Structures of OTC and DOX.

M NaCl ionic strength) were consistently maintained. For this study, oxytetracycline (OTC) and doxycycline (DOX) (Fig. 1) were chosen. With regard to the well-known fact that tetracyclines are "good" ligands for many metal ions, a series of metals were included in the study, mostly those which are present in a relatively high concentration, or play an important role, in the human body. In this paper the first results are presented for OTC and DOX with Zn^{2+}, Ca^{2+} and Mg^{2+} ions.

From the similar structures of OTC and DOX, see Fig. 1, similar chemical and biological properties can be expected. Administration of OTC and DOX, however, shows different biological effect and duration period. This fact cast doubt on the chemical expectation and lead to a comparative study.

Results on ternary complexes with OTC and DOX are also presented in which ascorbic acid was used as a secondary ligand. Vitamin C is generally present in considerable concentration in the blood and its possible influence on tetracycline complex formation was addressed in this study.

2. Experimental

OTC and DOX, both as HCl salts, were supplied by Chinoin Pharmaceutical Co., Hungary. Sodium chloride and ascorbic acid were purchased from Merck.

Potentiometric measurements were carried out by means of OP 211/1 digital pH meter (Radelkis, Hungary) equipped with an OP 0808P combined glass electrode. The standard NaOH solution was delivered from an OP 930 Automatic Burette (Radiometer). The ionic strength of each solution was adjusted with NaCl to 0.15 M to ensure isotonicity. Titrations were performed in a double-jacketed cell controlled at 37 ± 0.02°C with a Lauda M3 thermostat. High purity nitrogen was constantly bubbled through the solutions during the experiments.

For each titration, a 20 ml solution containing either the ligand or the ligand and metal ion was titrated with 0.15 M standard NaOH solution. A constant excess (0.5 mM) of hydrochloric acid was added to each solution prior to titration. The concentration ratio of the ligand to metal ion was 1:1 during the entire study employing 0.5 mM concentrations of each.

For the evaluation of the results, an iterative computer program (PSEQUAD) was applied [24]. When opalescence was observed, titration was continued up to the basic pH range but these points were not used for calculation.

3. Results and discussion

3.1. Parent complexes

The following protonation constants were obtained and used for calculations. For OTC: log $K_1 = 8.80 \pm 0.04$, log $K_2 = 7.20 \pm 0.05$ and log $K_3 = 3.22 \pm 0.06$ (literature data measured at identical temperture and ionic strength are 8.67, 7.11 and 3.22 respectively [9–12]). For DOX: log $K_1 = 9.16 \pm 0.04$, log $K_2 = 7.73 \pm 0.05$ and log $K_3 = 3.38 \pm 0.06$ (literature data are 8.68, 7.41 and 3.10 respectively [9–12]). The constants for OTC are in good correlation with those from the literature. For DOX, however, quite different values were obtained from those in the literature. This may imply that DOX samples were from a different source but may also reflect a difference in the actual ligand concentration used. (Throughout the

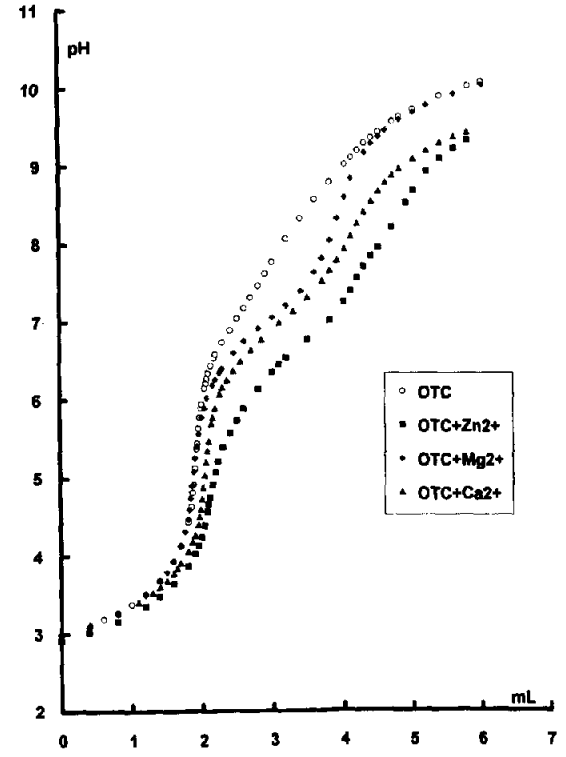

Fig. 2. pH-metric titration of OTC in the absence of (○) and presence (■: zinc; ◆: magnesium; ▲: calcium) of metal ions. $c_{OTC} = c_{Metal} = 0.5$ mM.

paper, A^{2-} refers to the fully deprotonated ligand and M^{2+} denotes the metal ions.)

According to the Calvin-type titration curves for OTC (Fig. 2), zinc ion forms complexes which differ from those of either calcium of magnesium. Indeed, as the calculated stability constants show (Table 1), zinc forms a 1:1 complex with OTC (MAH^+) in which one proton is still on the ligand. (In Tables 1–4, "pH range" refers to the section of the titrations where no heterogeneous phase was observed.) For Ca^{2+} and Mg^{2+}, no

Table 1
Calculated equilibrium constants (log K) for OTC (A^{2-})–metal ion (M^{2+}) complexes

Metal ion	Species	log K	pH range
Zn^{2+}	MAH^+	14.19 ± 0.07	3.96–6.35
Mg^{2+}	MA	5.77 ± 0.03	3.70–9.15
Ca^{2+}	MA	6.44 ± 0.09	3.95–8.00

Table 2
Calculated equilibrium constants (log K) for DOX (A^{2-})–metal ion (M^{2+}) complexes

Metal ion	Species	log K	pH range
Zn^{2+}	MA	8.20 ± 0.05	4.00–6.76
	MAH^+	15.02 ± 0.04	
Mg^{2+}	MA	6.82 ± 0.04	4.30–8.60
	MAH^+	13.68 ± 0.09	
Ca^{2+}	MAH^+	14.05 ± 0.07	3.91–6.54

such species could be detected. This observation is in line with that of Brion and co-workers [9,10].

When DOX was titrated with the metal ions (Fig. 3), calcium exhibited a different behavior from the other two metals. As Table 2 shows, both MA and MAH^+ species are formed with Zn^{2+} and Mg^{2+} but only the MAH^+ species was found to be present for calcium. (In contrast, literature data show the existence of an MA complex for calcium [10,11].)

These results indicate that OTC and DOX differ in terms of their chemistry, even though their structures are similar. The absence of the C6–OH group in DOX can cause a change in the binding characteristics, mainly in the protonation state of the complexes. This may indicate a different binding site of a certain metal ion to the tetracyclines. Reviewing the literature data, the assignments of the binding sites of the metal ions are quite different. From this study, the MAH^+ complex seems to have the proton on the

Table 3
Calculated equilibrium constants (log K) for ascorbic acid (B^-)–metal ion (M^{2+}) complexes

Metal ion	Species	log K	pH range
Zn^{2+}	MB^+	8.20 ± 0.09	3.20–7.30
	MBH^{2+}	15.66 ± 0.08	
Mg^{2+}	MB^+	6.57 ± 0.05	3.00–9.40
	MBH^{2+}	15.22 ± 0.05	
Ca^{2+}	MB^+	6.72 ± 0.04	3.00–9.12
	MBH^{2+}	15.42 ± 0.04	

Table 4
Calculated equilibrium constants (log K) for OTC or DOX (A^{2-}) and ascorbic acid (B^-)–metal ion (M^{2+}) complexes

Ligand	Metal ion	Species	log K	pH range
OTC	Zn^{2+}	MAB^-	14.83 ± 0.06	4.30–8.30
		MABH	22.24 ± 0.09	
	Mg^{2+}	MAB^-	12.00 ± 0.05	4.30–9.30
		MABH	20.45 ± 0.08	
	Ca^{2+}	MAB^-	12.74 ± 0.09	3.50–8.90
DOX	Zn^{2+}	$MABH^-$	22.65 ± 0.04	3.60–6.90
		$MABH_2^+$	29.24 ± 0.06	
	Mg^{2+}	MAB^-	14.17 ± 0.07	
		MABH	22.49 ± 0.07	4.00–8.90
		$MABH_2^+$	29.33 ± 0.10	
	Ca^{2+}	MABH	22.39 ± 0.05	3.95–6.65

dimethylammonium group which has been considered to be the most basic functional group of the tetracyclines. Therefore, the metals can be attached either to the tricarbonyl-methane (A ring) or the phenol-diketone (BCD ring) areas. When no proton is associated with the complex, the metal can be positioned on any of the three possible binding sites. The overall charge is 0 and +1 for the MA and MAH complexes respectively, which might explain the controversial literature data: a small change in the pH can affect the liquid–solid phase equilibrium that alters the final outcome of the calculation (both the species distribution and the stability constants).

3.2. Mixed ligand complexes

Ascorbic acid (the deprotonated form is denoted as B^-) is an important biomolecule and also acts as a ligand due to its en-diol structure. According to the authors' measurements, it has a log K value of 4.13 ± 0.04. Vitamin C forms both MB^+ and MBH^{2+} complexes with Zn^{2+}, Ca^{2+} and Mg^{2+} (see Table 3). The constants are of comparable magnitude to those of tetracyclines.

When both ligands (one of the tetracyclines, ligand A^{2-}, and ascorbic acid, ligand B^-) were in solution with the metals, various species could be detected, see Table 4. It is generally true that complexes with less protons are present for OTC than for DOX (e.g. even $MABH_2^+$ species were found with zinc and magnesium). Typically, the constants are similar for species of the same composition, regardless of the nature of the metal ion. This suggests that the tetracycline binding site might be the same for the metals (possibly the phenol-diketone which offers a chelate). However, Mg–OTC–ascorbic acid (MAB^-) has considerably less stability than all the other MAB^- complexes. The ternary complex results show a significant effect of Vitamin C on the metal complex formation of both OTC and DOX. Since a high intake of Vitamin C is a generally accepted way of maintaining good physical health and is strongly recommended in cases of sickness, these chemical results indicate a possible change in the biological effect of tetracyclines if they are administered together with Vitamin C.

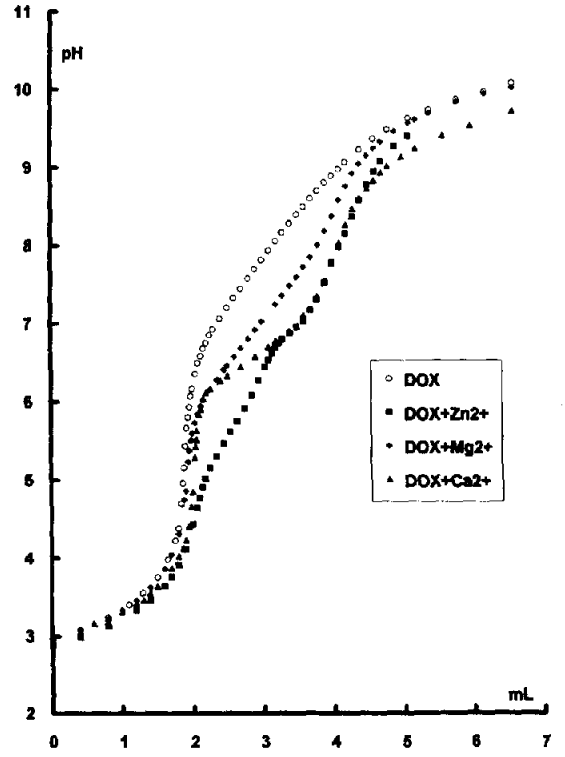

Fig. 3. pH-metric titration of DOX in the absence (○) and presence (■: zinc; ◆: magnesium; ▲: calcium) of metal ions. $c_{DOX} = c_{Metal} = 0.5$ mM.

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