



## METAL COMPLEXES OF TETRACYCLINES

### II. The use of macroscopic ionization constants in the calculation of the stability constants of metal complexes

*It is demonstrated that it is mathematically equivalent to use macroscopic or microscopic ionization constants for the calculation of the stability constants of metal complexes, provided that in the complexes formed the metal is coordinated to a unique chelation site although this may vary from metal to metal.*

The calculation of stability constants of the metal complexes of a given polybasic ligand from data obtained by potentiometric titrations requires the previous knowledge of the ionization constants of the protonated forms of the ligand.

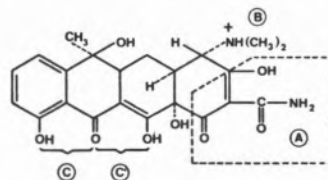
However, in some cases, those ionization constants do not correspond to the real processes which occur in solution and represent average values for a series of simultaneous equilibria, involving more than one reactive center. This happens when the ligand has several sites of similar basicity and the protons may ionize from those different sites in proportions which vary with the degree of neutralization.

The average values are usually referred to by «macroscopic» ionization constants and those corresponding to the real individual steps by «microscopic» ionization constants.

This is exactly the case for the tetracycline antibiotics, for which one obtains three macroscopic ionization constants corresponding to the ionization of the protonated species  $\text{LH}_3^+$ .

The values of  $\text{p}K$  are of the order of 3, 7 and 9 and can be easily calculated from potentiometric data, but there is no general agreement on what concerns the individual processes which origin such values.

STEPHENS, MURAI, BRUNINGS and WOODWARD (1) proposed that the three  $\text{p}K$  values represent the successive ionization of protons from the carboxamide (A), dimethylammonium (B) and  $\beta$ -diketone (C, C') moieties of the protonated tetracycline (I).



I: Protonated tetracycline

On the other hand, LEESON, KRUEGER and NASH (2) suggested that the order of ionization of the second and third groups should be reversed, basing this argument on the analogy with several ammonium quaternary salts.

Still other authors offered their own explanations but it wasn't until the work of RIGLER, BAG, LEYDEN, SUDMEIER and REILLEY (3), who analysed the state of protonation of the molecules for several degrees

of neutralization using a nuclear magnetic resonance technique, that a better understanding of the process was possible. These authors were able to demonstrate the existence of several simultaneous equilibria and calculated «microscopic» constants for each one of the individual twelve steps.

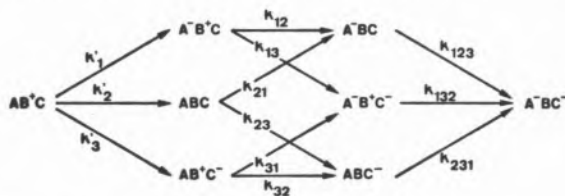
In these conditions one may ask if the method of calculating stability constants of metal complexes using data obtained by potentiometric titrations and involving the previous knowledge of the proton ionization constants is indeed legitimate, since macroscopic constants are normally used and the several simultaneous steps usually ignored. This is what actually happened in all the studies carried on metal complex formation by the tetracycline antibiotics and it became necessary to see whether the results are affected by this procedure or in which conditions can it be adopted.

In the present work we demonstrate that it is mathematically equivalent to use macroscopic or microscopic ionization constants, provided that in the complexes formed the metal is coordinated to an unique chelation site, although it may vary from metal to metal.

This hypothesis is supported by some experimental studies. Indeed, CONNOVER (4) suggested that the normal site for metal chelation is the  $\beta$ -diketone group  $C_{11}$ - $C_{12}$ , on the basis of a compared study of UV and visible spectra of the metal complexes formed with oxytetracycline and several model compounds. On the other hand, BAKER and BROWN (5), based on results of studies of reflectance spectra, proposed two oxygen atoms of the system 1, 2, 3 — tricarbonylmethane, one belonging to the amide group and the other to one of the hydroxyl groups  $C_1$  or  $C_3$ , as the donor atoms in the antibiotic molecule.

It may be added that whatever the site, it is reasonable to assume that it is unique and well defined, since the affinity of the metals for the several donors which exist in the molecule of the tetracyclines is considerably different and it is not to be expected that simultaneous equilibria are established, as it happens with the proton. As an example, one could recall the fact that ions with an inert gas electronic configuration have a very low affinity for nitrogen donors and it is unlikely that they can coordinate to the dimethylammonium or to the amide groups.

If the hypothesis is correct, the demonstration of the equivalence of macroscopic and microscopic constants is quite straightforward; the several possible mechanisms for the ionization of the protonated tetracyclines are as follows:



II: Microscopic acid-base equilibria according to REILLEY et al. (3).

It should be noticed that according to REILLEY *et al.*, group C' is always protonated and does not take any part in the equilibria which were considered.

In these conditions, the species present in solution will be:

$H^+$ ,  $OH^-$ ,  $K^+$ ,  $Cl^-$ ,  $NO_3^-$ ,  $AB^+C$ ,  $A^-B^+C$ ,  $ABC$ ,  $AB^+C^-$ ,  $A^-BC$ ,  $A^-B^+C^-$ ,  $ABC^-$  and  $A^-BC^-$

$$[LH_3^+] = [AB^+C]$$

$$[LH_2] = [A^-B^+C] + [ABC] + [AB^+C^-]$$

$$[LH^-] = [A^-BC] + [A^-B^+C^-] + [ABC^-]$$

$$[L^{2-}] = [A^-BC^-]$$

Twelve microscopic ionization constants will have to be considered, and these are defined as

$$k_1' = \frac{[A^-B^+C][H^+]}{[AB^+C]}$$

$$k_2' = \frac{[ABC][H^+]}{[AB^+C]}$$

$$k_3' = \frac{[AB^+C^-][H^+]}{[AB^+C]}$$

$$k_{12} = \frac{[A^-BC][H^+]}{[A^-B^+C]}$$

$$k_{13} = \frac{[A^-B^+C^-][H^+]}{[A^-B^+C]}$$

$$\begin{aligned}
 k_{21} &= \frac{[A^- BC][H^+]}{[ABC]} \\
 k_{23} &= \frac{[ABC^-][H^+]}{[ABC]} \\
 k_{31} &= \frac{[A^- B^+ C^-][H^+]}{[AB^+ C^-]} \\
 k_{32} &= \frac{[ABC^-][H^+]}{[AB^+ C^-]} \\
 k_{123} &= \frac{[A^- BC^-][H^+]}{[A^- BC]} \\
 k_{132} &= \frac{[A^- BC^-][H^+]}{[A^- B^+ C^-]} \\
 k_{231} &= \frac{[A^- BC^-][H^+]}{[ABC^-]}
 \end{aligned}$$

For the protonated tetracycline these constants were determined by REILLEY *et al.* (3) using a NMR technique and the values of the corresponding pk's are as follows.

$$\begin{array}{ll}
 pk_1' = 4.49 & pk_{23} = 7.29 \\
 pk_2' = 5.40 & pk_{31} = 7.55 \\
 pk_3' = 5.45 & pk_{32} = 7.24 \\
 pk_{12} = 8.00 & pk_{123} = 9.11 \\
 pk_{13} = 8.51 & pk_{132} = 8.60 \\
 pk_{21} = 7.09 & pk_{231} = 8.92
 \end{array}$$

The mass balances are:

$$\begin{aligned}
 Ca &= [AB^+ C] + [A^- B^+ C] + [ABC] + \\
 &+ [AB^+ C^-] + [A^- BC] + [A^- B^+ C^-] + \\
 &+ [ABC^-] + [A^- BC^-] + [ML]
 \end{aligned}$$

$$Cm = [M] + [ML]$$

$$[K^+] = a Ca$$

$$[NO_3^-] = 2 Cm$$

$$[Cl^-] = Ca$$

where Ca is the total concentration of the ligand tetracycline in any form and Cm is the total concentration of metal ion  $M^{2+}$ . The condition of electroneutrality, considering KOH as the titrant is:

$$\begin{aligned}
 [AB^+ C] + 2[M^{2+}] + [H^+] + a Ca &= \\
 &= [A^- BC] + [A^- B^+ C^-] + [ABC^-] + \\
 &+ 2[A^- BC^-] + [OH^-] + 2[M^{2+}] + 2[ML] + \\
 &+ [AB^+ C] + [A^- B^+ C] + [ABC] + [AB^+ C^-] + \\
 &+ [A^- BC] + [A^- B^+ C^-] + [ABC^-] + \\
 &+ [A^- BC^-] + [ML]
 \end{aligned}$$

By convenient rearrangement

$$\begin{aligned}
 a Ca + [H^+] - [OH^-] &= 2[A^- BC] + \\
 &+ 2[A^- B^+ C^-] + 2[ABC^-] + 3[A^- BC^-] + \\
 &+ [A^- B^+ C] + [ABC] + [AB^+ C^-] + 3[ML]
 \end{aligned} \quad (2)$$

If equation 1 is multiplied by 3 and equation 2 subtracted, one obtains

$$\begin{aligned}
 (3 - a) Ca - [H^+] + [OH^-] &= 3[AB^+ C] + \\
 &+ 2[A^- B^+ C] + 2[ABC] + 2[AB^+ C^-] + \\
 &+ [A^- BC] + [A^- B^+ C^-] + [ABC^-]
 \end{aligned} \quad (3)$$

The concentration of the different species may be expressed in terms of the microscopic ionization constants:

$$\begin{aligned}
 [AB^+ C] &= \frac{[A^- B^+ C][H^+]}{k_1'} = \frac{[A^- BC][H^+][H^+]}{k_{12} k_1'} = \\
 &= \frac{[A^- BC^-][H^+]^2 [H^+]}{k_{123} k_{12} k_1'} = \frac{[A^- BC^-][H^+]^3}{k_{123} k_{12} k_1'} \\
 [A^- B^+ C] &= \frac{[A^- BC][H^+]}{k_{12}} = \frac{[A^- BC^-][H^+]^2}{k_{123} k_{12}} \\
 [ABC] &= \frac{[A^- BC][H^+]}{k_{21}} = \frac{[A^- BC^-][H^+]^2}{k_{123} k_{21}} \\
 [AB^+ C^-] &= \frac{[ABC^-][H^+]}{k_{32}} = \frac{[A^- BC^-][H^+]^2}{k_{231} k_{32}} \\
 [A^- BC] &= \frac{[A^- BC^-][H^+]}{k_{123}}
 \end{aligned} \quad (1)$$

$$[A^- B^+ C^-] = \frac{[A^- BC^-][H^+]}{k_{132}}$$

$$[ABC^-] = \frac{[A^- BC^-][H^+]}{k_{231}}$$

If these concentrations are substituted in equation 3, one obtains

$$\begin{aligned} (3 - a) \text{ Ca} - [H^+] + [OH^-] = & 3 \cdot \frac{[A^- BC^-][H^+]^3}{k_{123} k_{12} k_1'} + 2 \cdot \frac{[A^- BC^-][H^+]^2}{k_{123} k_{12}} + \\ & + 2 \cdot \frac{[A^- BC^-][H^+]^2}{k_{123} k_{21}} + 2 \cdot \frac{[A^- BC^-][H^+]^2}{k_{231} k_{32}} + \\ & + \frac{[A^- BC^-][H^+]}{k_{123}} + \frac{[A^- BC^-][H^+]}{k_{132}} + \\ & + \frac{[A^- BC^-][H^+]}{k_{231}} \end{aligned}$$

And rearranging:

$$\begin{aligned} (3 - a) \text{ Ca} - [H^+] + [OH^-] = & [A^- BC^-] \left\{ 3 \frac{[H^+]^3}{k_{123} \cdot k_{12} \cdot k_1'} + 2 \frac{[H^+]^2}{k_{123} \cdot k_{12}} + \right. \\ & + 2 \frac{[H^+]^2}{k_{123} \cdot k_{21}} + 2 \frac{[H^+]^2}{k_{231} \cdot k_{32}} + \frac{[H^+]}{k_{123}} + \\ & \left. + \frac{[H^+]}{k_{132}} + \frac{[H^+]}{k_{231}} \right\} \end{aligned} \quad (4)$$

Equation 4 is formally identical to that obtained for calculating  $[L^{2-}]$  when ML complexes are formed (6) and if macroscopic constants are to be used instead of microscopic constants it is necessary that:

$$\begin{aligned} 3 \frac{[H^+]^3}{k_{123} \cdot k_{12} \cdot k_1'} + 2 \frac{[H^+]^2}{k_{123} \cdot k_{12}} + 2 \frac{[H^+]^2}{k_{123} \cdot k_{21}} + \\ + 2 \frac{[H^+]^2}{k_{231} \cdot k_{32}} + \frac{[H^+]}{k_{123}} + \frac{[H^+]}{k_{132}} + \frac{[H^+]}{k_{231}} = \\ = 3 \frac{[H^+]^3}{k_1 \cdot k_2 \cdot k_3} + 2 \frac{[H^+]^2}{k_2 \cdot k_3} + \frac{[H^+]}{k_3} \end{aligned}$$

where  $k_1$ ,  $k_2$  and  $k_3$  are the macroscopic ionization constants.

One should then have

$$\frac{1}{k_{123}} + \frac{1}{k_{132}} + \frac{1}{k_{231}} = \frac{1}{k_3}$$

$$\frac{1}{k_{123} \cdot k_{12}} + \frac{1}{k_{123} \cdot k_{21}} + \frac{1}{k_{231} \cdot k_{32}} = \frac{1}{k_2 \cdot k_3}$$

$$\frac{1}{k_{123} \cdot k_{12} \cdot k_1'} = \frac{1}{k_1 \cdot k_2 \cdot k_3}$$

But

$$\begin{aligned} \frac{1}{k_3} = \frac{[LH^-]}{[L^{2-}][H^+]} = \frac{[A^- BC]}{\underbrace{[A^- BC^-][H^+]}_D} + \\ + \frac{[A^- B^+ C]}{\underbrace{[A^- BC^-][H^+]}_E} + \frac{[ABC^-]}{\underbrace{[A^- BC^-][H^+]}_F} \end{aligned}$$

$$\begin{aligned} \frac{1}{k_2 \cdot k_3} = \frac{[LH_2]}{[L^{2-}][H^+]^2} = \frac{[A^- B^+ C]}{\underbrace{[A^- BC^-][H^+]^2}_A} + \\ + \frac{[ABC]}{\underbrace{[A^- BC^-][H^+]^2}_B} + \frac{[AB^+ C^-]}{\underbrace{[A^- BC^-][H^+]^2}_C} \end{aligned}$$

$$\frac{1}{k_1 \cdot k_2 \cdot k_3} = \frac{[LH_3^+]}{[L^{2-}][H^+]^3} = \frac{[AB^+ C]}{[A^- BC^-][H^+]^3}$$

$$k_{123} \cdot k_{12} \cdot k_1' = \frac{[A^- BC^-][H^+]}{[A^- BC]}$$

$$\cdot \frac{[A^- BC][H^+]}{[A^- B^+ C]} \cdot \frac{[A^- B^+ C][H^+]}{[AB^+ C]}$$

Hence

$$\frac{1}{k_{123} \cdot k_{12} \cdot k_1'} = \frac{[AB^+ C]}{[A^- BC^-][H^+]^3} = \frac{1}{k_1 \cdot k_2 \cdot k_3}$$

q. e. d.

$$\begin{aligned} k_{123} \cdot k_{12} = \frac{[A^- BC^-][H^+]}{[A^- BC]} \cdot \frac{[A^- BC][H^+]}{[A^- B^+ C]} = \\ = \frac{[A^- BC^-][H^+]^2}{[A^- B^+ C]} \end{aligned}$$

$$\frac{1}{k_{123} \cdot k_{12}} = \frac{[A^- B^+ C]}{[A^- BC^-][H^+]^2} = A$$

$$\begin{aligned} k_{123} \cdot k_{21} &= \frac{[A^- BC^-][H^+]}{[A^- BC]} \cdot \frac{[A^- BC][H^+]}{[ABC]} = \\ &= \frac{[A^- BC^-][H^+]^2}{[ABC]} \end{aligned}$$

$$\frac{1}{k_{123} \cdot k_{21}} = \frac{[ABC]}{[A^- BC^-][H^+]^2} = B$$

$$\begin{aligned} k_{231} \cdot k_{32} &= \frac{[A^- BC^-][H^+]}{[ABC^-]} \cdot \frac{[ABC^-][H^+]}{[AB^+ C^-]} = \\ &= \frac{[A^- BC^-][H^+]^2}{[AB^+ C^-]} \end{aligned}$$

$$\frac{1}{k_{231} \cdot k_{32}} = \frac{[AB^+ C^-]}{[A^- BC^-][H^+]^2} = C$$

$$A + B + C = \frac{1}{k_2 k_3}$$

q. e. d.

$$\frac{1}{k_{123}} = \frac{[A^- BC]}{[A^- BC^-][H^+]} = D$$

$$\frac{1}{k_{132}} = \frac{[A^- B^+ C^-]}{[A^- BC^-][H^+]} = E$$

$$\frac{1}{k_{231}} = \frac{[ABC^-]}{[A^- BC^-][H^+]} = F$$

$$D + E + F = \frac{1}{k_3}$$

q. e. d.

The purpose of this paper seems then to be fulfilled, e. g., it is possible to use macroscopic ionization constants instead of microscopic constants in the calculation of stability constants of metal complexes provided that there is only one chelation site in the molecule of the antibiotic for each metal, although it may vary from metal to metal.

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## RESUMO

Demonstra-se que é matematicamente equivalente o uso de constantes de ionização macroscópicas ou microscópicas no cálculo de constantes de estabilidade de complexos, desde que nestes o metal se coordene a uma única sede de quelatão, a qual pode, de resto, variar de metal para metal.