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**DETERMINATION OF THE STABILITY CONSTANTS FOR THE
COMPLEXES OF RARE-EARTH ELEMENTS AND TETRACYCLINE**

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DETERMINATION OF THE STABILITY CONSTANTS FOR THE COMPLEXES OF RARE-EARTH ELEMENTS AND TETRACYCLINE

M Saiki* and F. W. Lima

ABSTRACT

Stability constants for the lanthanide elements complexes with tetracycline were determined by the methods of average number of ligands, the two parameters and by weighted least squares. The technique of solvent extraction was applied to obtain the values of the parameters required for the determination of the constants.

INTRODUCTION

It has been shown that rare-earth elements and the antibiotic tetracycline hydrochloride (TC) form complex compounds extractable into benzyl alcohol^(12,14). Separation procedures for the lanthanides, uranium, thorium and scandium were devised using TC dissolved in benzyl alcohol, as extractant agent^(12,14). In this paper the determination of the stability constants for the rare-earth elements and TC was carried out for the 15 lanthanide elements (except promethium) using solvent extraction technique and labelled radioisotopes solutions for the determination of distribution ratios *D*. The constants were calculated by the methods of average number of ligands, two parameters and weighted least squares⁽²¹⁾.

Most of the works on complexes of tetracycline have been carried out along the lines of devising separation procedures for some metallic elements, preparation of complexes of radioelements for clinical applications and for determination of stability constants values of tetracycline and some metals in order to correlate these values with the biological activity of the ligand used as antibiotic⁽²⁰⁾.

Doluisio and Martin⁽⁵⁾ favoured the explanation that the biologically active derivatives form 2:1 complexes, i.e., two ligands for each metal ion of copper, nickel and zinc. The inactive derivatives would form 1:1 complexes. However, Benet and Goyan⁽³⁾ when determining the stability constants of tetracycline and the derivatives chlorotetracycline, dimethylchlorotetracycline, 4-epichlorotetracycline and 4-epianhydrotetracycline with copper ions, concluded that the biological inactive tetracyclines form complexes with two ligand molecules for each copper ion.

Stability constants for the complexes of the various tetracycline and bivalent ions of Mg, Ca, Sr, Ba, Mn, Co, Ni, Cu, Zn and Cd were determined by Silva and Dias^(19,20) who have shown that the bacteriostatic action of tetracycline is correlated with complex formation capability.

Albert and Rees⁽¹⁾ determined the stability constants for tetracycline and the trivalent ions Fe and Al and for the bivalent ions Cu, Ni, Fe, Co, Zn and Mn. Sakaguchi et al⁽¹⁷⁾, determined the constants for the ions Fe⁺³, Zr⁺⁴, Th⁺⁴, UO₂⁺², Al⁺³ and Mg⁺².

Complexes of tetracycline and the lanthanide elements have been used by Tubis and Morrison⁽²²⁾ for the examination of brain tumors. Such complexes allow the use of various radioisotopes with similar chemical and biochemical properties but with rather different nuclear characteristics such as half-lives and energy of emitted radiations.

(*) From a thesis submitted by M. Saiki to the Institute of Chemistry, University of São Paulo, in partial fulfillment for a Doctor of Science's Degree.

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THEORY

To calculate stability constants the following correlation⁽²¹⁾ was used

$$D = I_o/I_a = K_D \beta_3 [A]^3 / \sum_{n=0}^3 \beta_n [A]^n \quad (1)$$

in which D is the distribution ratio of the lanthanide; I_o and I_a are the activities of the radioelements in the organic and aqueous phases, respectively; K_D is the distribution constant of the complex MA_3 , [A] is the concentration of the ligand ion in the aqueous phase and β_n ($n=1,2,3$) are the formation constants for the complexes MA , MA_2 , MA_3 (charges are omitted for simplicity)

By using labelled radioelements the concentrations of the metal can be made much smaller than the initial concentration of the ligand. The amount of ligand in the complex molecule can thus be ignored and the following mass balance correlation will prevail.

$$V_o C_o = [HA]_o V_o + ([HA] + [A]) V \quad (2)$$

V_o and V are the volumes of organic and aqueous phases, respectively; C_o is the initial concentration of tetracycline in the organic phase; $[HA]_o$ is the concentration of tetracycline in the organic phase, after equilibrium; $[HA]$ and $[A]$ are the concentrations of the ligand in the aqueous phase, also after equilibrium (organic phase is indicated by sub-index o; no sub-indexes in the concentration formulae indicate aqueous phase). For the pH values used in the present work (smaller than 5.50) tetracycline is taken as a monoacid⁽⁴⁾, that is the dissociation of the second and third hydrogen is considered as very small.

Equation (2) can be rearranged to give

$$V_o C_o = [A] [H] (1 + K_a / [H]) (V_o D' / V + 1) V / K_a \quad (3)$$

in which D' is the distribution ratio for the ligand and K_a its first ionization constant, i.e.,

$$D' = [HA]_o / ([HA] + [A]) \text{ and } K_a = [H][A] / [HA]$$

From (3) one has

$$pA = pK_a - pH - \log C_o / (1 + K_a [H]^{-1}) (1 + D' V_o V^{-1}) V / V_o \quad (4)$$

Calculation of the Stability Constants

a) Average Number Method

The application of the average number method or Bjerrum's method, was made through the use of the equations⁽²¹⁾

$$\bar{n} = 3 + d \log D / dpA \quad (5)$$

and

$$\bar{n} + \beta_1 [A] (\bar{n} - 1) + \beta_2 [A]^2 (\bar{n} - 2) + \beta_3 [A]^3 (\bar{n} - 3) = 0 \quad (6)$$

\bar{n} is the average number of ligands. Experimental values of \bar{n} and $[A]$ substituted in equation (6) will give a linear system of equations through which the values of the constants β_1, β_2 and β_3 can be determined

b) Two Parameters Method

The Dyrssen-Sillén method of two parameters was applied by using the following equations⁽²¹⁾

$$\log \beta_n = a_n + b_n (3 - n) \quad (7)$$

The parameters **a** and **b** are defined by

$$\log \beta_3 = 3a \text{ and } \log k_n - \log k_{n+1} = 2b = 2 \log \alpha \quad (8)$$

$k_n (n = 1, 2, 3)$ are the consecutive stability constants. The graph of $(\log D - \log K_D)$ versus $\log y = \log [A] + a$ was prepared using the following equation for various values of **b**:

$$\log D = \log K_D + 3 \log y - \log \sum_{n=0}^3 y^n \alpha^{n(3-n)} \quad (9)$$

By placing the templet so obtained over the experimental curve of $\log D$ versus pA , the value of **b** is determined from the best curve in the templet that coincides with the experimental curve. **a** is equal to pA for $\log y = 0$, for the best curve indicated previously by using the templet. The values of **b** and **a** are then used in (7) for calculation of β_n

c) Least Squares Method

Equation (1) can be rearranged to give

$$Z = \sum_{n=0}^3 a_n [A]^n \quad (10)$$

in which

$$Z = [A]^3 D^{-1} \text{ and } a_n = \beta_n / K_D \beta_3 \quad (11)$$

Equation (10) is solved for the a_n values by the method of weighted least squares using digital computers. In this method the parameters a_n are calculated in such a way as to minimize R in

$$R = \sum_{i=0}^i w_i (\sum_{n=0}^i a_n [A]_i^n - Z_i)^2 \quad (12)$$

The weight w_i is taken as being

$$w_i = 1/\sigma_i^2 \quad (13)$$

in which σ_i^2 is the variance of the measurement Z_i . For calculation of the variance, the errors taken into account are the ones in the values of the distribution ratio D and in the values of the concentration of free ligand $[A]$. This last error was taken as being equal to the error in the measurement of the pH of the aqueous phase⁽¹⁶⁾. Errors in the determination of the distribution ratio D were calculated by means of the standard deviations of counting of organic and aqueous phase, taken as the square root of the accumulated number of counts divided by the counting time.

To solve equation (10) by the least squares method a program for the IBM/370 Model 155 computer was prepared⁽¹⁸⁾. When the parameters a_n occur with negative signs or when their standard deviations are larger than the corresponding a_n values, the computer program discards such values for a_n and recalculates a new set of values for the remaining a_n . In these cases the corresponding values of β_n are not reported, but only the product of the consecutive constants are registered. The values of β_n and k_n as function of a_n are

$$\beta_n = a_n / a_0 \quad (14)$$

$$k_n = a_n / a_{n-1} \quad (15)$$

EXPERIMENTAL

Preparation of Solutions

Tetracycline Solutions

Fresh TC (Laborerápica Bristol) solutions were prepared every day since the ligand molecules decompose in aqueous solution at room temperature. Solutions were used within six hours after preparation. Analytical grade benzyl alcohol (Carlo Erba) was used as organic phase for dissolution of TC. The organic solvent was previously equilibrated with distilled water from a quartz apparatus and vice-versa, aqueous phase was previously equilibrated with benzyl alcohol.

Lanthanide Solutions

The lanthanide oxides (La and Ce from the British Drug House Ltd and the other lanthanides from Johnson Matthey Chemicals Ltd) were irradiated in a thermal neutron flux corresponding to 5×10^{12} n/cm² sec for a time from half an hour to eight hours, depending of the radioelement to be obtained. After irradiation the oxides were dissolved with drops of analytical grade perchloric acid (Merck) at 20%, with gentle heating and then diluted to obtain a final concentration corresponding to 2.0×10^{-4} M in the lanthanide elements. Dissolution of cerium oxide required some drops of hydrogen peroxide (Baker P A), besides the perchloric acid.

Sodium perchlorate stock solutions were prepared by adding perchloric acid to sodium hydroxide (Carlo Erba P.A.) solutions.

Buffer solutions for pH standardization were prepared in accordance with Lange⁽⁸⁾, using potassium biphthalate (Baker P.A.) and potassium chloride (Baker P.A.).

Complex Formation and Extraction

To show that the metal extracted into the organic phase is bonded to the tetracycline molecule, the radioactive lanthanide solution at ionic strength corresponding to 0.10 M in NaClO_4 was contacted with pure benzyl alcohol at various pH values. No activity was detected in the organic phase (benzyl alcohol only) showing that the extractable species was the lanthanide-tetracycline complex.

Type of Complexes Formed

In order to check if polynuclear complexes would be formed, the distribution ratios D were determined for various concentrations of the lanthanide element (terbium). Concentration of TC was maintained constant and the one for the lanthanide element was made to vary as function of pH.

Temperature was maintained at $25^\circ\text{C} \pm 0.5^\circ\text{C}$ and ionic strength corresponded to 0.10 M in NaClO_4 . Results are presented in Figure 1. A statistical F test applied to the data showed that all curves, for the four different concentrations of terbium, are coincident at a confidence level of 95%. This means that the distribution ratios D are independent of the concentration of the metallic element in the interval range of 10^{-5} to 10^{-4} M and that only mononuclear complexes are formed⁽²¹⁾.

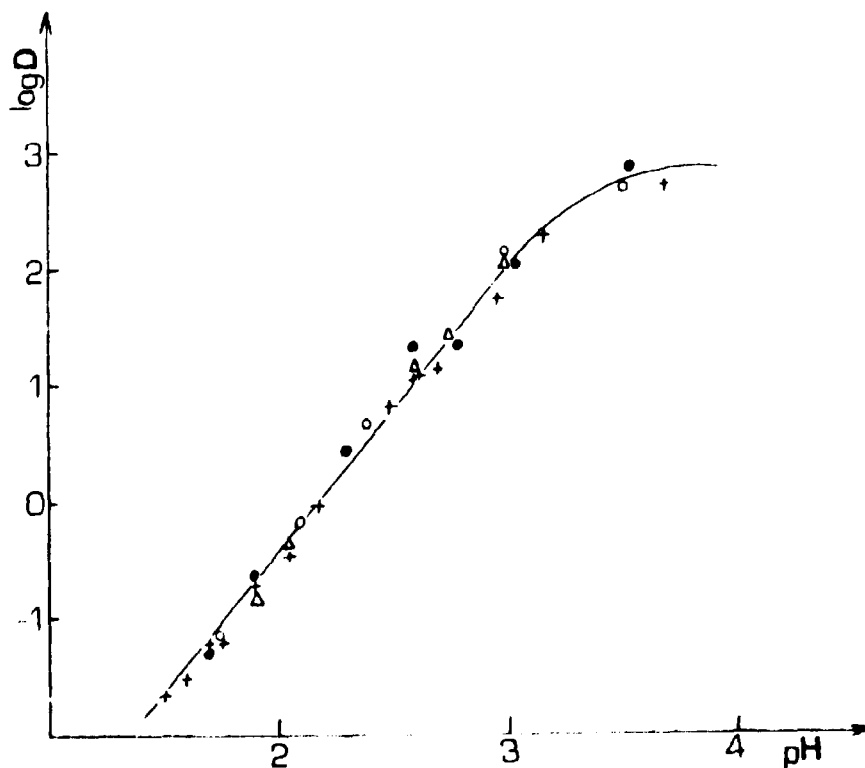


Figure 1 - Distribution ratio of Tb versus pH. $[\text{TC}] : C_0 = 0.010 \text{ M}$; $[\text{Tb}]$: (Δ) $1.0 \times 10^{-5} \text{ M}$; (+) $2.0 \times 10^{-5} \text{ M}$; (o) $4.0 \times 10^{-5} \text{ M}$; (•) $1.0 \times 10^{-4} \text{ M}$.

The ionization constants for TC, required for the calculation of pA values by formula (4) were determined by potentiometry at $25^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and ionic strength corresponding to 0.10 M in NaClO_4 , Figure 2. In table I are presented the results obtained as well as literature values. D' values, also required for the calculation of pA values, were determined by spectrophotometric measurements of the distribution of TC between benzyl alcohol and aqueous solution of NaClO_4 , 0.10 M, Figure 3.

Table I
Macroscopic Ionization Constants for Tetracycline

pK_{a1}	pK_{a2}	pK_{a3}	Ionic Strength (μ)	Temperature $^{\circ}\text{C}$	References
3.35	7.82	9.57	0.01 M	20	(3)
3.30	7.68	9.69	0	25	(9)
3.33	7.75	9.61	*	25	(9)
3.42	7.52	9.07	0.1 M KNO_3	25	(19)
3.69	7.63	9.24	0.01 M	30	(5)
3.33	7.70	9.50	0.01 M	25	(3)
3.39	7.44	8.85	0.10 M NaClO_4	25	(this work)

* not indicated

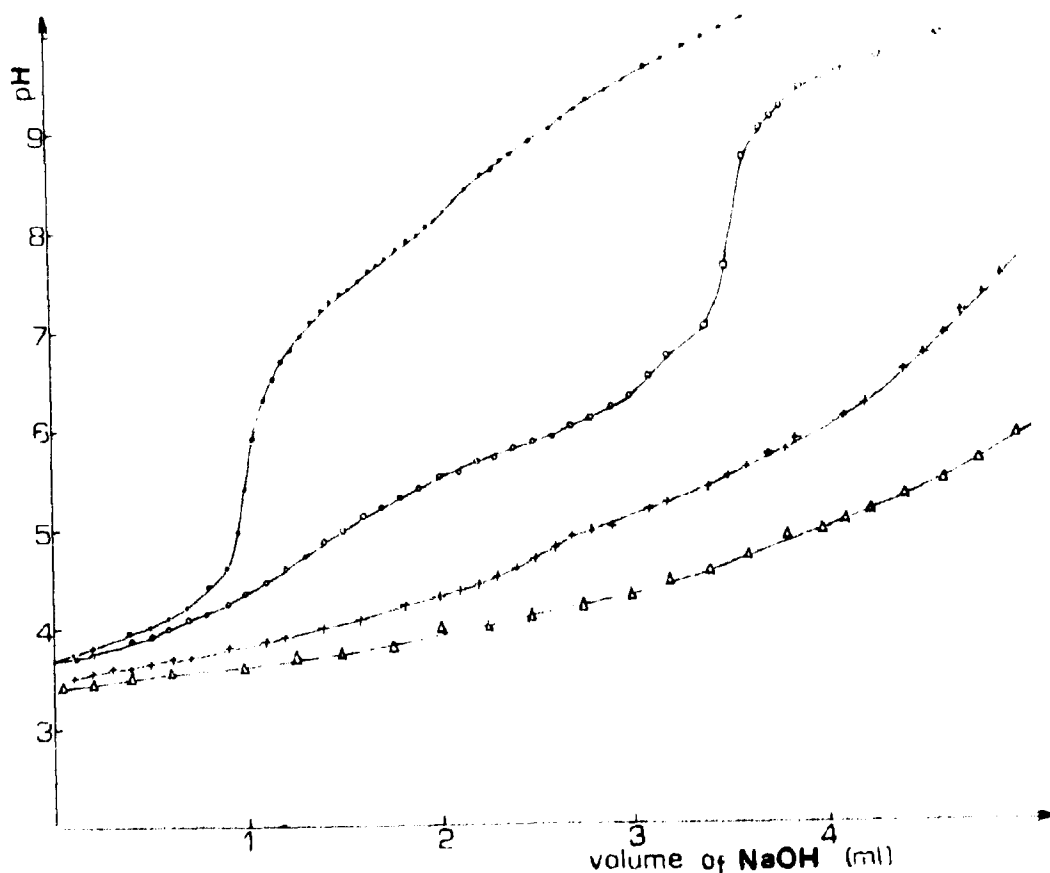


Figure 2 - Potentiometric titration of TC 0.010 M with NaOH 0.010 M. Relation La-TC: (e) no La; (o) 1:1; (+) 1:2; (Δ) 1:3.

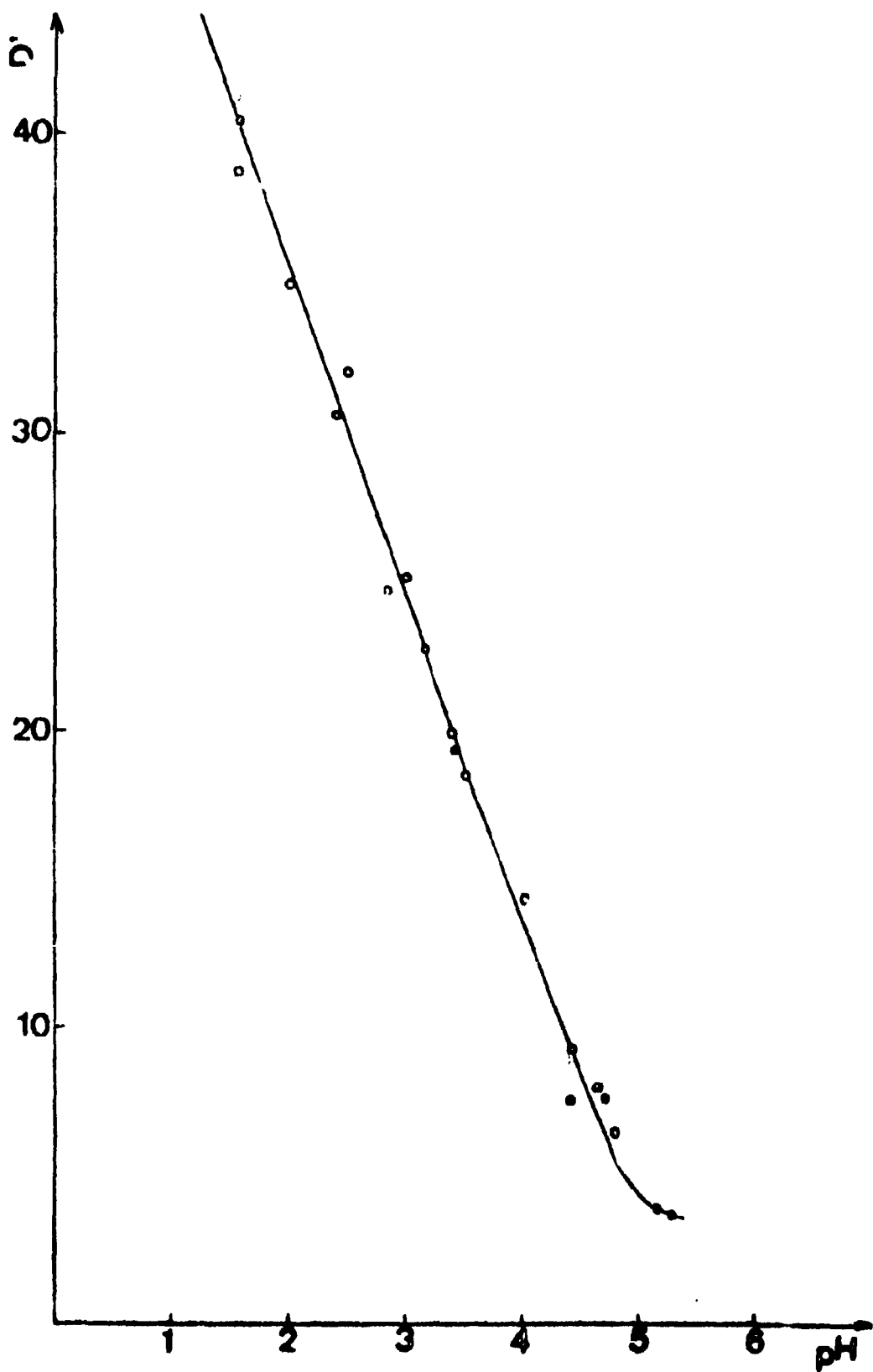


Figure 3 - Distribution ratio of TC versus pH.

Eventual formation of complexes with the formula $MA_n(OH)_p$ and $MA_n(OH)_p(HA)_r$ and negatively charged complexes besides the mononuclear species MA_n , was checked by determining the distribution ratios D for terbium as function of pA for two different concentrations of TC. Results are presented in Figure 4. From Figure 4 it is seen that the distribution ratio D varies with pA and it is independent of the initial TC concentrations, that is, the distribution follows equation (1), indicating non-existence of hydroxycomplexes, negatively charged complexes or complexes of the type $MA_n(OH)_p(HA)_r$ ⁽²¹⁾. The same set of experiments were carried out for europium and thulium with the same results indicating the formation of complexes of the MA_n type. In fact hydroxycomplexes should not be formed, as verified, since hydrolysis of rare-earths ions does not occur at pH values smaller than 5.50^(10,11).

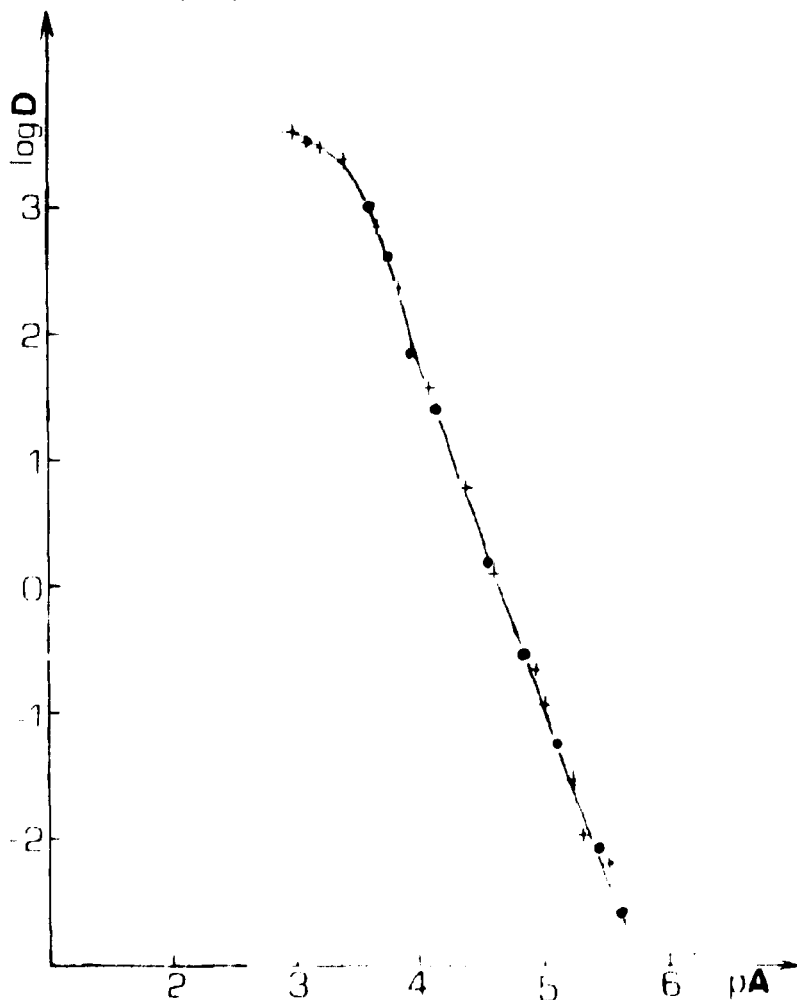


Figure 4 - Distribution ratio of Tb versus pA . $[Tb] = 2.0 \times 10^{-5} M$. $[TC]: C_0 = 0.010 M (+); 0.004 M (●)$.

The formation of the MA_n type of complexes was crosschecked by conductimetric titrations of tetracycline and lanthanum. Experiments were carried out at the temperature of $25^\circ C \pm 0.5^\circ C$. Total volume of solution to be titrated was equal to 30 ml. The titrating solution was added in volume fractions equal to 1 ml. Results are indicated in Figure 5 for the element lanthanum. Curve A shows the formation of 1:1 species (MA) and 1:2 (MA_2). In curve B it is shown the formation of 1:1 and 1:3 species.

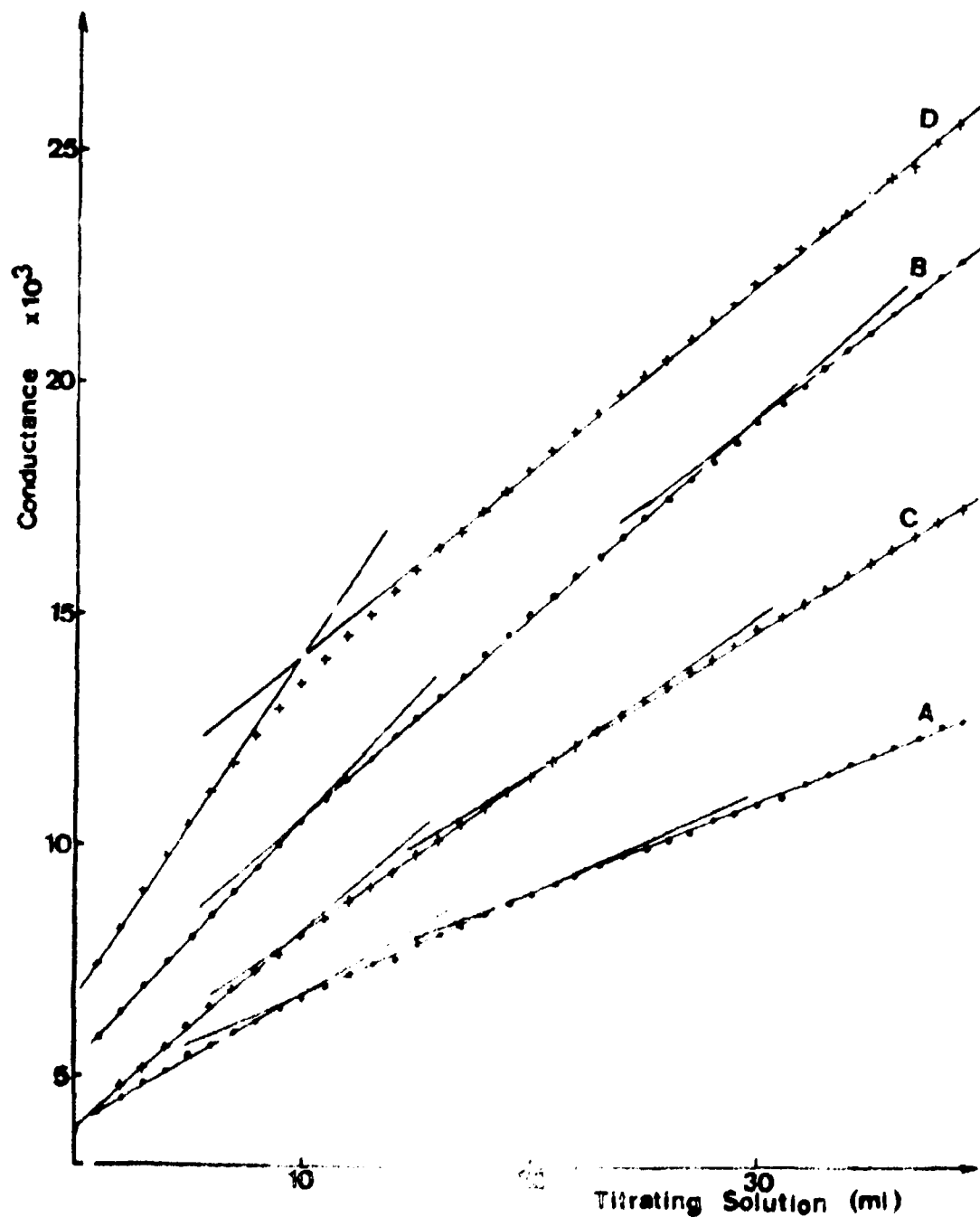


Figure 5 - Conductimetric titrations of TC and ATC. Curves A and C: 10 ml of LaCl_3 , 0.010 M + 20 ml of water. Titrating solutions: TC 0.010 M(A) and ATC 0.010 M (C). Curves B and D: 30 ml of TC solution 0.010 M (B); 30 ml of ATC solution 0.010 M(D). Titrating solution: LaCl_3 0.010 M.

Complexation Position in the Tetracycline Molecule

TC has three ionizable hydrogens corresponding to the tricarbonyl-methane group, $pK_{a1} = 3.30$; phenolic diketone, $pK_{a2} = 7.68$; and dimethylamino, $pK_{a3} = 9.63$ ⁽⁹⁾

Complexation position for the lanthanide ions was determined by potentiometric and conductimetric titrations, as well as by infrared absorption spectrophotometry. Results for TC potentiometric titration with sodium hydroxide solutions, in presence and in absence of lanthanum ions are shown in Figure 2. It can be seen that the increase of pH on titrating TC-La is less pronounced than when titration is carried out in absence of lanthanum. This indicates formation of the complex by the tricarbonylmethane group, since this group is the one that dissociates at pH values smaller than 5.50.

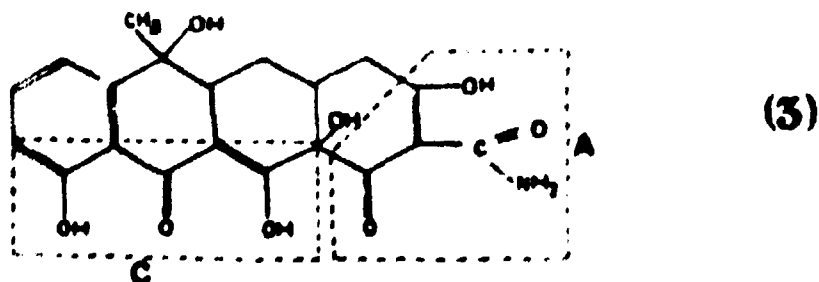
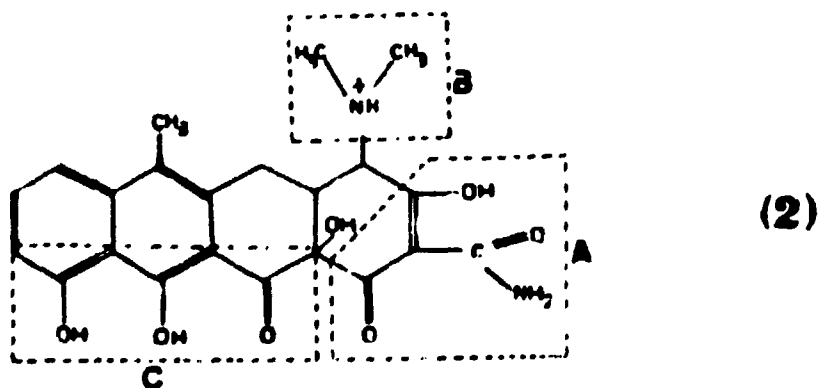
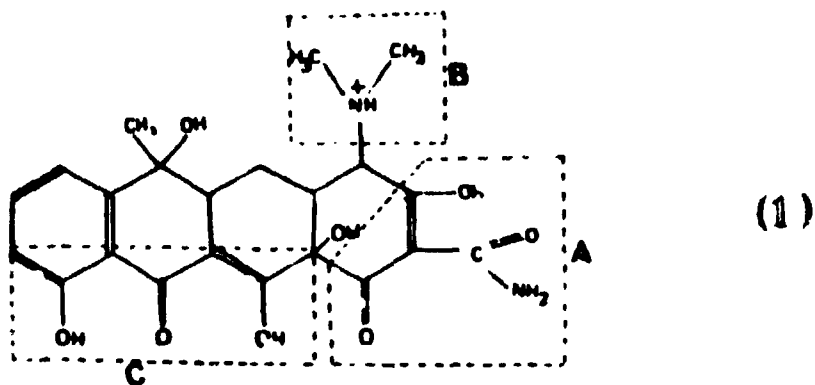


Figure 6 - (1) TC; (2) ATC; (3) DTC

For conductimetric titrations, two tetracycline derivatives (anhydrotetracycline ATC and dedimethylamine-tetracycline DTC, Figure 6) were used as well as the TC ligand. TC and ATC were titrated in aqueous solutions and DTC in methanol since its solubility in water is rather small. Formation of complexes with a relation metal-ligand equal to 1:1, 1:2 and 1:3 was thus confirmed, Figure 5 and 7. It is thus seen that the lanthanide is not coordinated through the nitrogen of group B (dimethylamino) since this group is not present in the DTC. The same conclusion was reached by solvent extraction technique since the compounds formed with ATC and DTC are both extractable into benzyl alcohol.

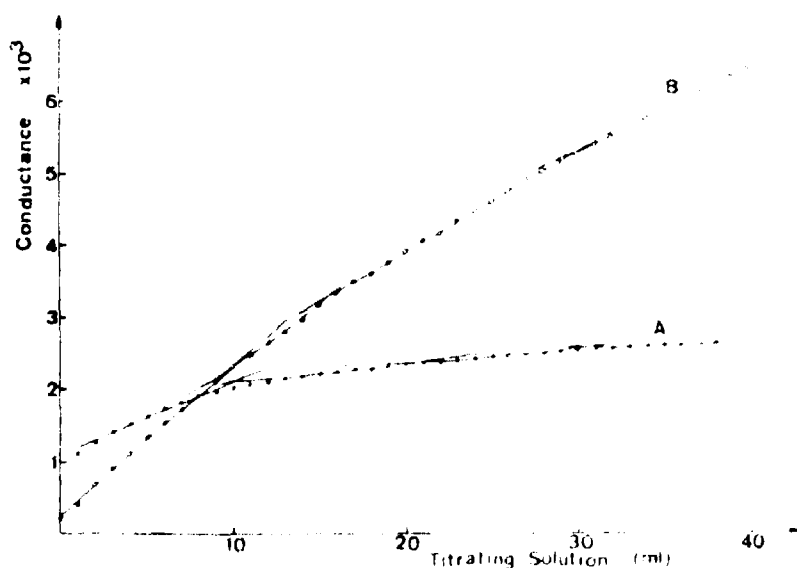


Figure 7 - Conductimetric titration of DTC.

- (A) : 10 ml of LaCl_3 0.010 M + 20 ml methanol. Titration with DTC 0.010 M.
 (B) : 30 ml DTC 0.010 M. Titration with LaCl_3 0.010 M.

Absorption infrared spectra of TC and of La-TC were made. Both compounds were emulsified in mineral oil (Nujol). The La-TC solid compound was prepared in accordance with Baker and Brown⁽²⁾. The spectra showed the following characteristics:

- TC and La-TC have the same absorption bands in the interval from 200 to 1500 cm^{-1} .
- The compound La-TC obtained at pH=3.0 and the one obtained at pH=4.5 are the same and both have water molecules in their structures.
- TC presents three absorption bands at 1580, 1610 and 1670 cm^{-1} which are not present in the spectrum of La-TC; instead a large band, at approximately 1600 cm^{-1} , is presented by La-TC (Figure 8). Since the absorption corresponding to the value at 1600 cm^{-1} is due to the vibration of the group $\text{C}=\text{O}$ it is seen that lanthanum is linked to the TC molecule by the tricarbonyl methane group A.

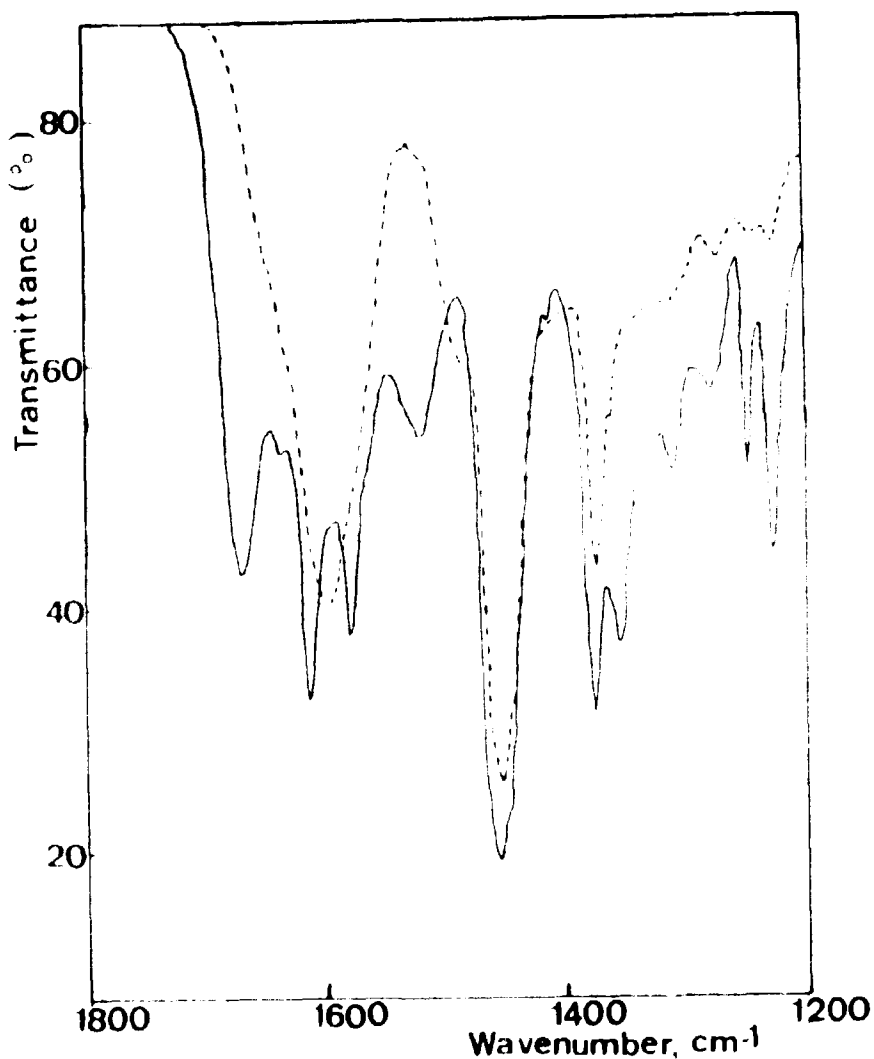


Figure 8 – Infrared absorption spectrum of TC and La-TC. Key : TC; La-TC.

Based on all information obtained from potentiometric and conductimetric titrations for TC, ATC and DTC, as well as the information from the absorption spectra in the infrared region for the solid compound La-TC, it is seen that the complexation of the lanthanide is through the hydrogen position of the tricarbonylmethane group in the TC molecule and whose thermodynamic ionization constant is $10^{-3.30}$, that is $pK_a = 3.30$, at 25°C .

Williamson and Everett⁽²³⁾ have also shown, by nuclear magnetic resonance, that the trivalent ions of Nd, Tb, La, V and the bivalent ones Cu, Mn, Co, Ca and Mg are linked to the TC molecule by tricarbonylmethane group.

Determination of Distribution Ratios

The extraction systems consisted of 5 ml of benzyl alcohol and 5 ml of aqueous labelled lanthanide solution with ionic strength corresponding to 0.10 M sodium perchlorate. Concentration of

TC in the organic solvent varied from 10^{-3} to 10^{-4} M and of the metal ion in the aqueous phase from 10^{-3} to 10^{-4} M. pH of the aqueous phase was adjusted by adding diluted solutions of perchloric acid or sodium hydroxide. pH measurements were carried out with a scale reading of 0.05 units. pH values in which extractions were made were always smaller than 5.50.

The phases were equilibrated at $25^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ by shaking in a mechanical stirrer for the desired time. Results were reproducible for a shaking time from a few minutes up to 10 hours, which was the maximum time at which equilibrium conditions were examined. A standard 30 minutes shaking time was adopted. After equilibration the phases were separated by centrifugation at 3500 rpm for 5 minutes. Aliquots of each phase were taken and counted using a well-type NaI(Tl) scintillation counter 7×7 cm, coupled to a multichannel analyser. The area under the main peaks for each radioisotope were evaluated by using a minicomputer on line with the analyser. Background counting was subtracted in each case and the net counting rate and the corresponding standard deviation were calculated by using a program in BASIC language. Each counting was reported to zero time.

RESULTS AND DISCUSSION

The curve of $\log D$ versus the atomic number of the lanthanide for a pH equal to 2.45 and initial TC concentration equal to 0.010 M is presented in Figure 9.

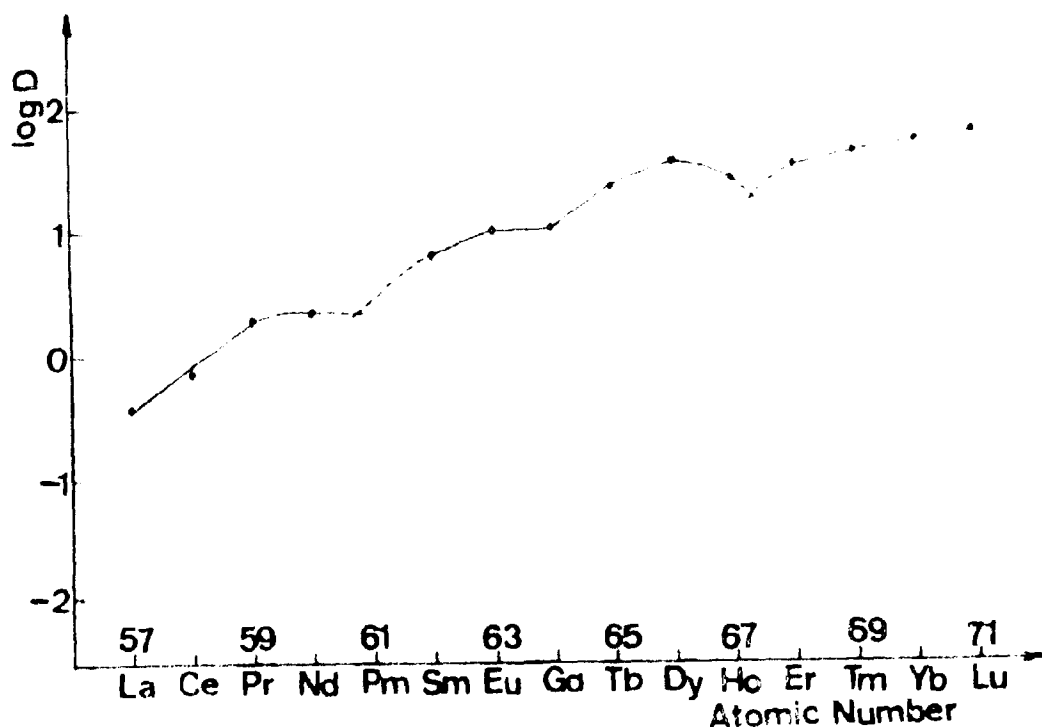


Figure 9 – $\log D$ versus atomic number of lanthanides.

From the graph of $\log D$ versus atomic number of the lanthanide elements presented in Figure 9, it is seen that, also for this system, the double-double effect (Fidelis and Siekierski⁽⁷⁾) or tetrad effect (Preppard et al⁽¹⁵⁾) is present. The sets of curves have, as usual, an intercept between neodymium and promethium; the second intercept is at gadolinium, which is a common point for the second and third part of the curves; the third intercepts lies between holmium and erbium. The positions of these intercepts are the same as for other ligands and the lanthanides^(7,15).

Values for the stability constants are presented in Tables II, III and IV. In these Tables β_n are the stability constants and k_n the consecutive equilibrium constants for the MA_n complexes. The constants β_n are expressed in units corresponding to $(\text{litre})^n / (\text{mol})^n$ and the constants k_n in units $(\text{litre}) / (\text{mol})$.

Table II
Stability Constants for the Complexes of Lanthanides
with Tetracycline Determined by the
Average Number Method

Element	$\log \beta_1$	$\log k_2 \cdot k_3$	$\log \beta_3$
La	2.82	6.08	8.90
Ce	3.10	6.32	9.42
Pr	3.59	6.52	10.11
Nd	3.62	6.22	9.84
Sm	3.45	6.78	10.23
Eu	3.53	6.38	9.91
Gd	3.60	6.64	10.24
Tb	3.78	6.81	10.59
Dy	3.72	6.82	10.54
Ho	3.53	6.88	10.41
Er	3.71	7.01	10.72
Tm	3.99	6.77	10.76
Yb	4.26	6.69	10.95
Lu	4.20	6.22	10.42

Table III
Stability Constants for the Complexes of Lanthanides
with Tetracycline Determined by the
Two Parameters Method

Element	$\log \beta_1$	$\log \beta_2$	$\log \beta_3$
La	2.95	5.90	8.85
Ce	3.10	6.20	9.30
Pr	3.25	6.50	9.75
Nd	3.20	6.40	9.60
Sm	3.30	6.60	9.90
Eu	3.35	6.70	10.05
Gd	3.35	6.70	10.05
Tb	3.45	6.90	10.35
Dy	3.45	6.90	10.35
Ho	3.50	7.00	10.50
Er	3.65	7.30	10.95
Tm	3.70	7.40	11.10
Yb	3.55	7.10	10.65
Lu	3.50	7.00	10.50

Table IV
Stability Constants for the Complexes of Lanthanides
with Tetracycline Determined by the
Least Squares Method

Element	$\log \beta_1$	$\log k_2 \cdot k_3$	$\log \beta_3$
La	3.50 ± 0.16	5.84 ± 0.26	9.35 ± 0.22
Ce	3.31 ± 0.24	6.16 ± 0.30	9.47 ± 0.22
Pr	2.86 ± 0.12	7.45 ± 0.24	10.32 ± 0.18
Nd	2.92 ± 0.38	6.67 ± 0.41	9.59 ± 0.15
Sm	2.81 ± 0.77	7.20 ± 0.78	10.01 ± 0.14
Eu	3.97 ± 0.14	5.93 ± 0.38	9.90 ± 0.37
Gd	3.79 ± 0.14	6.53 ± 0.17	10.32 ± 0.13
Tb	3.78 ± 0.19	6.73 ± 0.23	10.52 ± 0.15
Dy	2.70 ± 0.59	7.72 ± 0.59	10.43 ± 0.06
Ho	3.53 ± 0.33	7.26 ± 0.33	10.79 ± 0.12
Er	3.73 ± 0.21	7.14 ± 0.22	10.87 ± 0.11
Tm	4.26 ± 0.10	6.78 ± 0.12	11.05 ± 0.08
Yb	3.55 ± 0.35	7.33 ± 0.44	10.88 ± 0.27
Lu	4.48 ± 0.09	6.36 ± 0.11	10.84 ± 0.11

The calculation of $\log \beta_2$ by the least squares method, was not performed by the computer program since the value of a_2 was rejected by the program as a consequence of a too large standard deviation for a_2 or a negative value for a_2 . A new set of values for a_0 , a_1 and a_3 was recalculated by the computer program and in this way instead of β_2 values the product $k_2 k_3$ is reported. β_2 values were also not calculated by the average number of ligands since these values occurred with negative signs.

The omission of a_2 parameter in the least squares calculations does not necessarily means that the corresponding complex does not exist, but only that the stability constant cannot be calculated because of uncertainties in the corresponding experimental data, probably because the concentration of MA_2 is not high enough to influence the measurements.

The difficulty encountered in calculation β_2 by the average number of ligands may also supports the conclusion that the calculation of this value is dubious.

The two parameters method makes the assumption that the ratio of adjacent complexity constants is the same throughout a series of complexes of a metal ion M and a ligand A. This approximation has proved helpful in systems where the data are not accurate enough to allow a separate determination of all consecutive equilibrium constants, Dyrssen and Sillén⁽⁶⁾. The small concentration of MA_2 species would not give accurate enough data for application of the average number of ligands as well as for the application of least squares method, for β_2 determination, both the mentioned approximation of the two parameters method will be helpful for the evaluation of an approximate value for β_2 .

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RESUMO

São determinadas as constantes de estabilidade dos complexos dos elementos lantanídicos com o antibiótico tetraciclina. Os métodos usados foram o do número médio de ligantes, o de dois parâmetros e dos mínimos quadrados ponderados.

Foi aplicada a técnica de extração com solventes para obter os valores dos parâmetros necessários para determinação das constantes.

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