

Bis(2,4-dinitrophenyl) phosphate hydrolysis mediated by lanthanide ions[†]

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ABSTRACT: The kinetics of the hydrolysis of bis(2,4-dinitrophenyl) phosphate (BDNPP) were studied in basic solutions in the presence of La^{3+} , Sm^{3+} , Tb^{3+} and Er^{3+} . Bis-Tris propane (BTP) buffer was used to stabilize the Ln^{3+} hydroxide complexes in solution. Two equivalents of the 2,4-dinitrophenolate ion (DNP) were liberated for each equivalent of BDNPP and the reaction showed first-order kinetics. Potentiometric titrations showed the formation of dinuclear complexes such as $[\text{Ln}_2(\text{BTP})_2(\text{OH})_n]^{(6-n)}$, with values of n varying as a function of pH, for all studied metals. Hence the catalytic effect depends on the formation of dinuclear lanthanide ion complexes with several hydroxo ligands. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: bis(2,4-dinitrophenyl) phosphate; lanthanide(III); hydrolysis; catalysis; complex formation

INTRODUCTION

Phosphoryl group transfer reactions catalyzed by enzymes are central for the energy transfer process of all organisms. Studies of models for these reactions provide important mechanistic information for the understanding of phosphate hydrolysis reactions.^{1–3} Thus, investigations of the nucleophilic substitution mechanisms of phosphoric acid derivatives contribute to the understanding of the mechanisms in enzymatic transformations.^{1,4} The catalysis by transition metals and organometallic compounds is part of a wide range of investigations that are developing very quickly owing to its potential to model a variety of enzymatic processes.

The catalysis by metal ions is attributed to coordination of the substrates and stabilization of the transition state.⁴ Among the metal ions studies, the lanthanide ions have attracted great attention owing their excellent catalytic effects in hydrolysis and nucleophilic substitution reactions at phosphoric esters.^{5–8} Lanthanides can either act as free ions or associate with nucleophilic species, resulting in large incremental increases in reaction rate.^{9,10} The total charge of the metal ion and its ability to polarize P—O bonds are important factors in the study of the mechanisms of reactions promoted by metals. The lanthanide ions present some advantages over transition metals in catalytic efficiency, mainly owing to the effect of the charge, high coordination numbers and low binding directionality.^{11,12}

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This paper reports investigations on the participation of lanthanide ions in hydrolysis reactions of the activated aryl diester bis(2,4-dinitrophenyl) phosphate.

EXPERIMENTAL

Materials

Bis(2,4-dinitrophenyl) phosphate (BDNPP) (**1**) was prepared as described previously.¹³ Stock solutions of BDNPP ($10^{-3} \text{ mol l}^{-1}$) were prepared in dry CH_3CN and the final reaction mixture contained 0.05% (v/v) of CH_3CN . Stock solutions containing $1.0 \times 10^{-3} \text{ mol l}^{-1}$ of Ln^{3+} cations (La^{3+} , Sm^{3+} , Tb^{3+} and Er^{3+}) were prepared by cautious addition of concentrated HClO_4 up to complete neutralization of the respective carbonates and standardized by inductively coupled plasma mass spectrometry (ICP-MS) on a Perkin-Elmer SCIEX ELAN 6000 spectrometer. Bis-Tris propane {1,3-bis[tris(hydroxymethyl)methylamino]propane (BTP)} buffer from Sigma was used as a ligand to stabilize the lanthanide ions in solution. Deionized water was used in all measurements and was boiled, bubbled with nitrogen and kept under a nitrogen atmosphere to avoid the presence of carbon dioxide.

Kinetics

The hydrolysis reaction of the BDNPP was followed by monitoring the increase in absorbance at 400 nm due to the formation of the 2,4-dinitrophenoxide ion (DNP). All absorbance measurements were made on a HP 8453 diode array UV–visible spectrophotometer with thermostated

cell holder. Reaction solutions were prepared by mixing appropriate amounts of metal and the BTP ligand stock solutions to the desired volume and the pH was adjusted by adding small volumes of acid or base solutions as necessary, using a HANNA Model 200 pH-meter. To prevent metal hydroxide precipitation, the kinetics were studied with excess of BTP over the lanthanide ions. The reactions were initiated by adding an aliquot of the substrate stock solution. All reactions gave strict first-order kinetics over more than five half-lives. Observed first-order rate constants (k_{obs}) were estimated from linear plots of $\ln(A_{\infty} - A_t)$ against time (t) by using an iterative least-squares program from Microcal Origin 5.0 software; the correlation coefficients, r , were >0.996 for all kinetic runs. All reactions were followed at 25 °C. The pH values of the solutions after kinetic runs were measured and did not show significant variations. The reaction order regarding the metal was studied at pH 8.5 and the kinetic measurements were realized by varying the concentration of metal ions from 1.0×10^{-4} to $1.0 \times 10^{-3} \text{ mol l}^{-1}$.

Potentiometry

Potentiometric titrations were performed following the general recommendations of Martell and Motekaitis.¹⁴ All titrations were accomplished in a 100 ml thermostated cell kept under nitrogen at 25 °C. The initial volume of titrating solution was 50 ml and the ionic strength was kept constant with NaClO_4 . Measurements of pH were made after small additions of carbonate-free NaOH solution. The software BEST7 was used to calculate all equilibrium constants and species distributions were calculated by using SPE software and plotted with the SPEPLOT program.¹⁴ Titrations of BTP and its mixtures with lanthanide ions were performed in the concentration range $0.001\text{--}0.01 \text{ mol l}^{-1}$ of each component.

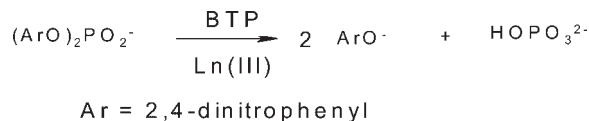
Products

The only reaction products, 2,4-dinitrophenol and inorganic phosphate, were identified from absorption and NMR spectra, with comparison with the spectra of authentic material. All NMR spectra were monitored on a Varian Unity Inova 400 spectrometer in D_2O at 25 °C, generally with a delay time of 1 s.

RESULTS AND DISCUSSION

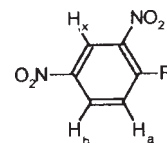
Hydrolysis of BDNPP

Addition of any of the Ln^{3+} metal ions (La^{3+} , Sm^{3+} , Tb^{3+} and Er^{3+}) promoted a fast BDNPP hydrolysis reaction and we found that, at the end of reaction, 1



Scheme 1

Table 1. NMR spectral data for products of the reaction of BDNPP hydrolysis by different Ln^{3+} complexes with BTP at 25 °C



Compound	^1H NMR δ (ppm)	^{31}P NMR δ (ppm)
DNP-	6.74 (d, 1H, $J = 9.6 \text{ Hz}$, Ar), 8.11 (dd, 1H, $J_{\text{ab}} = 9.6 \text{ Hz}$ and $J_{\text{bx}} = 3.0 \text{ Hz}$, Ar), 8.90 (d, 1H, $J = 3.0 \text{ Hz}$, Ar)	
Pi		2.17

equiv. of inorganic phosphate and 2 equiv. of 2,4-dinitrophenoxide ion are formed (Scheme 1, Table 1).

Figure 1 shows the pH–rate constant profiles for BDNPP hydrolysis in the presence of the metal ions. In all cases, the observed first-order rate constants (k_{obs}) increase steeply above pH 6, reaching a pH-independent region. The principal increase in the reactivity is observed in the range between pH 6.0 and 8.0, where with a change of just two pH units the rate constants of the reaction increase by a factor of ca 200 for La^{3+} , 105 for Sm^{3+} , 290 for Tb^{3+} and 330 for Er^{3+} . The pH dependence is consistent with the formation of hydroxo complexes at high pH values, which are the catalytically active species.

Figure 2 shows the values of k_{obs} as a function of Ln^{3+} ion concentration for the BDNPP hydrolysis reaction at a constant pH of 8.5. The observed rate constant is dependent on the Ln^{3+} concentration, and k_{obs} increases as a function of $[\text{Ln}^{3+}]$, showing saturation above $6 \times 10^{-4} \text{ mol l}^{-1}$ for all Ln^{3+} ions. The profiles are indicative of a catalytic process which involves complex formation between BDNPP and the lanthanides, with a fairly high binding constant and more than one metal ion involved in the reaction.

The results obtained from the pH and metal concentration effects over the observed rate constant of BDNPP hydrolysis suggest that the observed hydrolysis reaction is promoted by cooperative interactions between hydroxide groups and lanthanide ions, as suggested previously.^{7,15,16} Scheme 2 shows a reaction scheme where K_{ass} corresponds to the formation constant of the intermediate Ln^{3+} –BDNPP complex formed between the lanthanide ion complex **1** and BDNPP, and k_{obs}

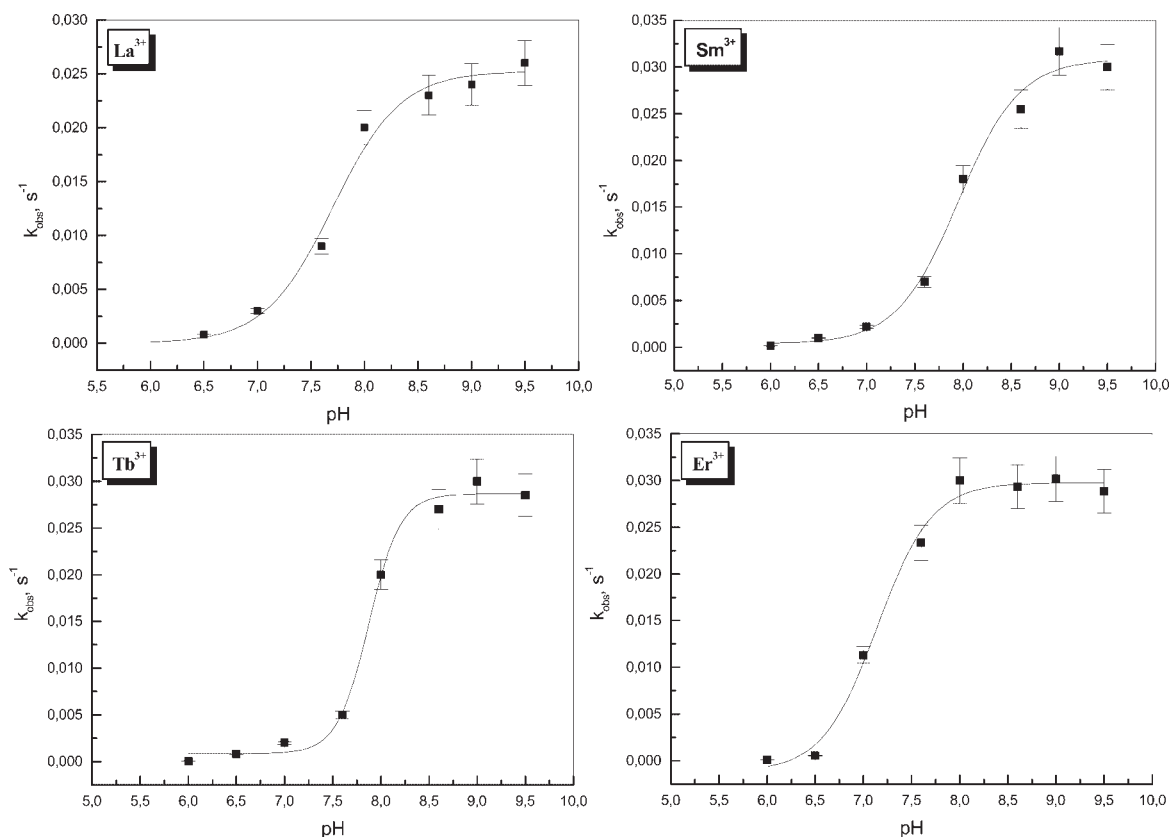


Figure 1. Observed rate constants of the hydrolysis of BDNPP at 25 °C in the presence of $1.0 \times 10^{-3} \text{ mol l}^{-1} \text{ Ln}^{3+}$ and $1.0 \times 10^{-2} \text{ mol l}^{-1}$ BTP as a function of pH

corresponds to the observed first-order rate constant for the hydrolysis of BDNPP in the presence of complex **1**. In Fig. 2, saturations are observed at rather low metal concentrations. If, as usual, the saturation is due to binding of the substrate to catalyst, K_{ass} values in the range 1600–2500 M^{-1} can be obtained, and the calculated K_{ass} values are similar to the reported formation constants of an intermediate substrate–lanthanide complex for the hydrolysis of 4-nitrophenyl phosphate.⁷ In the literature such saturation kinetics have been reported, e.g. by Liu and Schneider¹⁷ with lanthanide aquo ions and by Chin *et al.* with Ln(III)–hydrogen peroxide.¹⁶

The hypothetical structure of complex **1** (Scheme 2) was proposed by Yatsimirsky and co-workers based on potentiometric data and the electrospray ionization mass spectra of Ln^{3+} –BTP complexes.⁷ Deprotonation of metal-bound water molecules in complex **1** is responsible for the formation of the reactive hydroxo complexes (see below). Clearly, complex formation followed by deprotonation of water molecules bonded to the Ln^{3+} –BTP complexes^{18,19} is important for the catalytic effect, and the species responsible for this catalysis are the hydroxo complexes. The exact stoichiometry and stability constants for the La^{3+} complex have been reported⁷ and those for Sm^{3+} , Tb^{3+} and Er^{3+} were determined by potentiometric titrations of the metal ions in the presence of BTP.

Equilibrium data for the Ln^{3+} –BTP system

Figure 3 shows potentiometric titration curves for $1 \times 10^{-3} \text{ mol l}^{-1}$ BTP solutions in the presence of 1 mol equiv. of lanthanide (Sm^{3+} , Tb^{3+} and Er^{3+}) perchlorates. Similar data for the La^{3+} complex were reported by Yatsimirsky and co-workers.⁷ Each curve represents a different experiment and its form gives information on the stoichiometry and stability of possible species formed in the solution. The curves shown in Fig. 3 present one inflection at an a value of 6, showing that the addition of 6 mol of NaOH per mole of metal ion is required to neutralize 6 mol of protons.

The equilibria shown in Scheme 3 involve the formation of dinuclear, penta-, hexa-, hepta- and octahydroxo species. They were used to fit the potentiometric titration data with the best standard deviations and the equilibrium constants for the corresponding reactions (first column in Table 2). These constants were calculated from the experimental data shown in Fig. 3, and the logarithms of the calculated equilibrium constants ($\log K$) are given in Table 2.

The fit of the potentiometric titration curves assuming the formation of mononuclear complexes was not satisfactory. Similar results in the literature for systems involving other metals of the lanthanide series⁷ agree with the results presented in this work, which demonstrates the

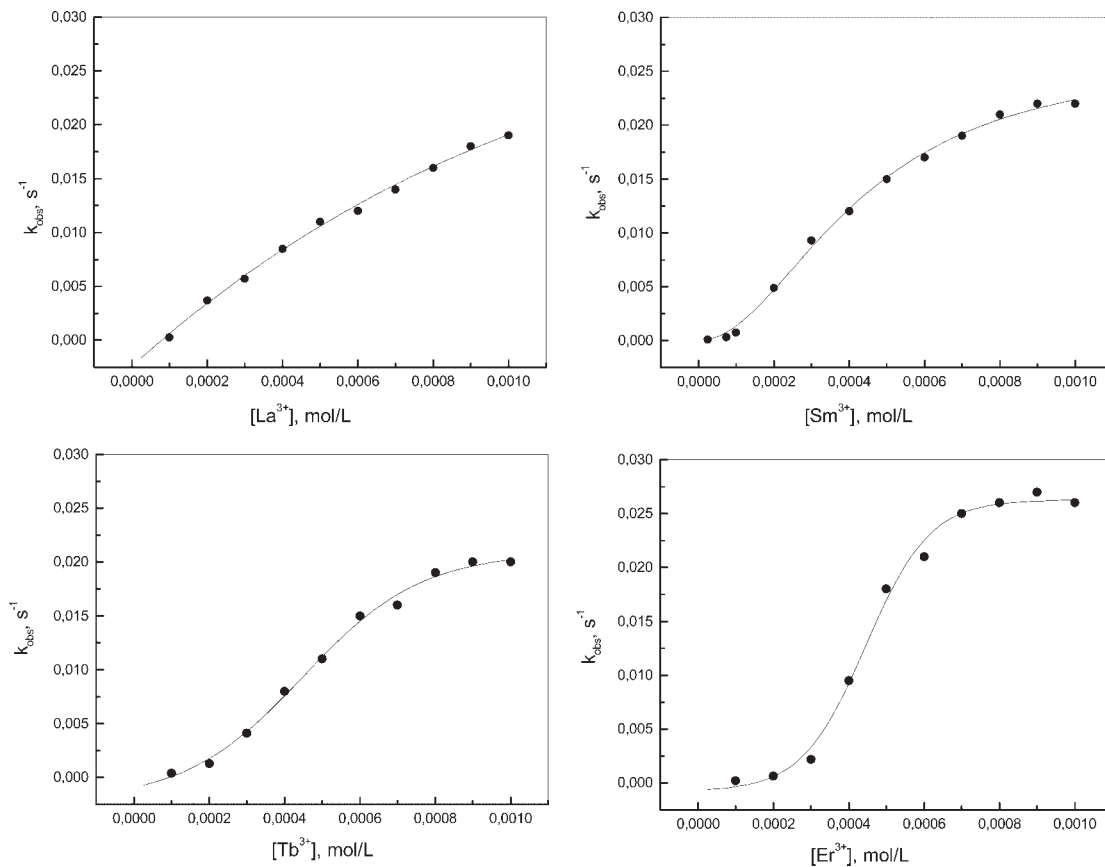
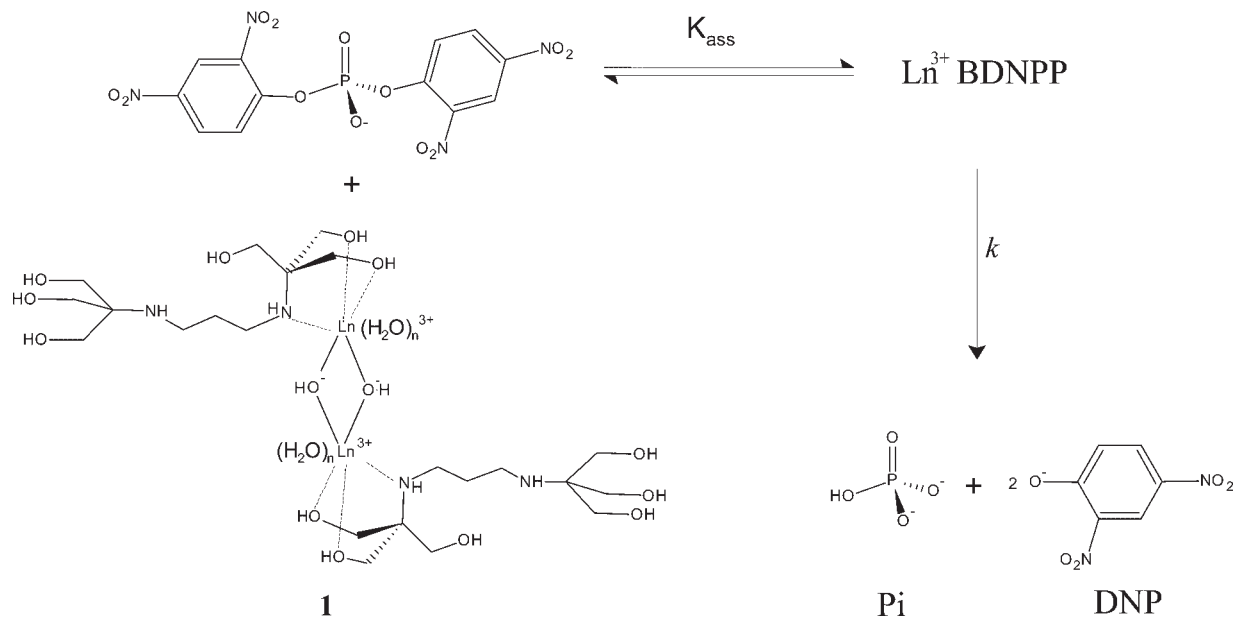


Figure 2. Dependence of the observed rate constants for the hydrolysis of BDNPP at 25°C in the presence of $1.0 \times 10^{-2} \text{ mol l}^{-1}$ BTP and pH 8.50 as a function of Ln^{3+} concentration



Scheme 2

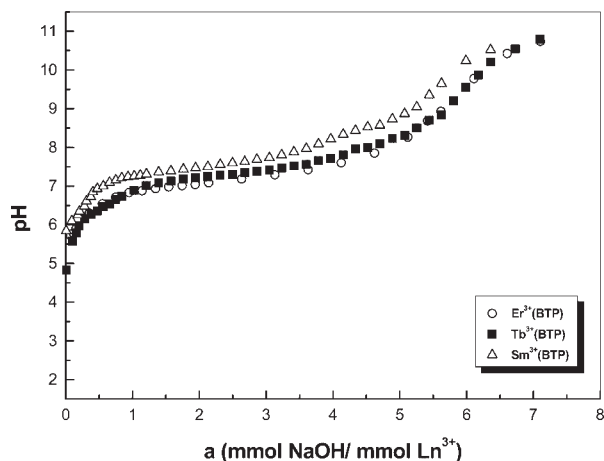
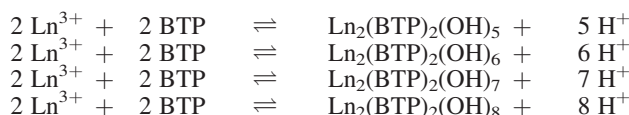


Figure 3. Titration curves of 0.001 mol l⁻¹ BTP-Ln³⁺ (Sm³⁺, Tb³⁺ and Er³⁺) perchlorate 1:1 mixtures in NaClO₄ at 25.0 °C; a is the number of added NaOH equivalents



Scheme 3

Table 2. Hydrolysis constants for the hydroxo complexes of Ln³⁺ with BTP at 25 °C at 0.1 mol l⁻¹ NaClO₄

Equilibrium	log K ^a		
	Sm ³⁺	Tb ³⁺	Er ³⁺
[BTPH]-[BTP][H]	9.05 ^b	—	—
[BTPH ₂]-[BTPH][H]	6.85 ^b	—	—
[Ln(OH)][H ⁺]-[Ln]	-8.39 ^c	-8.21 ^c	-8.04 ^c
[Ln ₂ (BTP) ₂ (OH) ₅][H ⁺] ⁵	-25.09	-23.83	—
-[Ln] ² [BTP] ²	—	—	—
[Ln ₂ (BTP) ₂ (OH) ₆][H ⁺] ⁶	—	-32.60	-28.76
-[Ln] ² [BTP] ²	—	—	—
[Ln ₂ (BTP) ₂ (OH) ₇][H ⁺] ⁷	-42.96	-40.50	—
-[Ln] ² [BTP] ²	—	—	—
[Ln ₂ (BTP) ₂ (OH) ₈][H ⁺] ⁸	-53.07	-49.43	-46.29
-[Ln] ² [BTP] ²	—	—	—

^a Log of the equilibrium constants for the equilibria in column 1.

^b Protonation constants for BTP.

^c Formation constants for monohydroxo complexes Ln³⁺ at 25 °C and ionic strength 0.5 mol l⁻¹.²⁰

formation of species involving the participation of two Ln³⁺ ions and two BTP molecules.

Chin *et al.*¹⁶ also observed the formation of La₂(OH)₅ species by potentiometric titration, which confirmed the strong tendency of the lanthanides to form dinuclear complexes in aqueous solution.

Figures 4, 5 and 6 show the species distribution diagrams for the Ln³⁺-BTP systems, for Sm³⁺, Tb³⁺ and Er³⁺ ions, respectively, together with the pH profiles of observed rate constant for the catalysis. It can be observed that for all metals the hydroxo complexes are the active species toward the hydrolysis reaction.

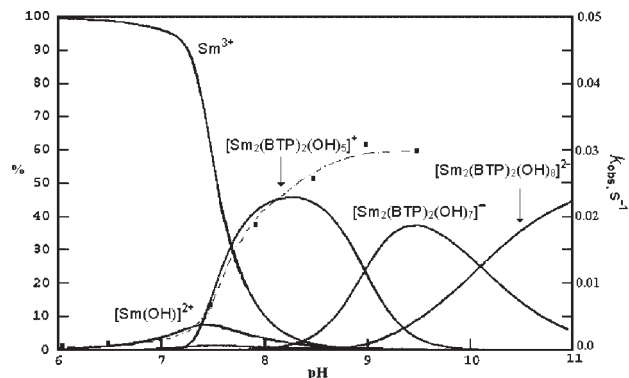


Figure 4. The solid lines represent the species distribution diagrams for hydroxo complexes of 0.001 mol l⁻¹ BTP and Sm³⁺ perchlorate 1:1 mixtures in NaClO₄ at 25.0 °C. The dashed line corresponds to the k_{obs} vs pH profiles at 25 °C in the presence of 1 × 10⁻³ mol l⁻¹ Sm³⁺ and 10⁻² mol l⁻¹ BTP

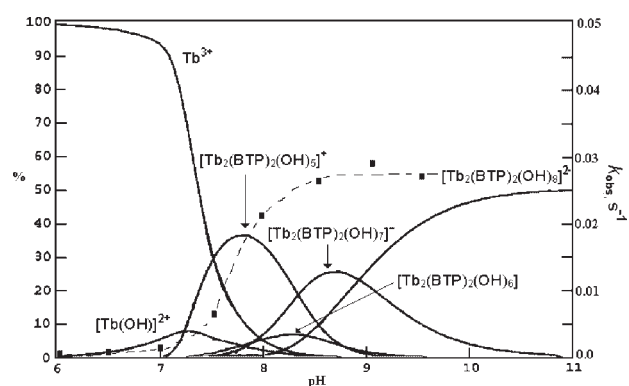


Figure 5. The solid lines represents the species distribution diagram for hydroxo complexes of 0.001 mol l⁻¹ BTP and Tb³⁺ perchlorate 1:1 mixtures in NaClO₄ at 25.0 °C. The dashed line corresponds to the k_{obs} vs pH profiles at 25 °C in the presence of 1 × 10⁻³ mol l⁻¹ Tb³⁺ and 10⁻² mol l⁻¹ BTP

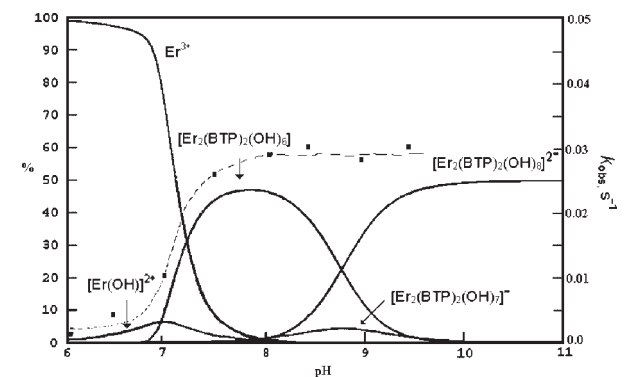


Figure 6. The solid lines represents the species distribution diagrams for hydroxo complexes of 0.001 mol l⁻¹ BTP and Er³⁺ perchlorate 1:1 mixtures in NaClO₄ at 25.0 °C. The dashed line corresponds to the k_{obs} vs pH profiles at 25 °C in the presence of 1 × 10⁻³ mol l⁻¹ Er³⁺ and 10⁻² mol l⁻¹ BTP

The results in Fig. 4 show that the [Sm₂(BTP)₂(OH)₅]⁺ complex reaches maximum formation (45.3%) at pH 8.2, which decreases at higher pH, whereas the [Sm₂(BTP)₂(OH)₇]⁻ species reach its maximum (37.9%) at

pH 9.4; above this pH, the predominant species is $[\text{Sm}_2(\text{BTP})_2(\text{OH})_8]^{2-}$.

Species distribution diagrams for Tb^{3+} -BTP show similar results to those for the Sm^{3+} system. The $[\text{Tb}_2(\text{BTP})_2(\text{OH})_5]^+$ complex reaches a maximum (36.5%) at pH 7.8, and at this pH there are also small amounts of the $[\text{Tb}_2(\text{BTP})_2(\text{OH})_6]$ and $[\text{Tb}_2(\text{BTP})_2(\text{OH})_7]^-$ complexes. The $[\text{Tb}_2(\text{BTP})_2(\text{OH})_7]^-$ species reaches its maximum at pH 8.7 with 25.6% formation, whereas the $[\text{Tb}_2(\text{BTP})_2(\text{OH})_6]$ species only reaches 6.9% at pH 8.3 (Fig. 5).

Figure 6 shows the formation of 47% of the $[\text{Er}_2(\text{BTP})_2(\text{OH})_6]$ complex at pH 7.8. The yield of the $[\text{Er}_2(\text{BTP})_2(\text{OH})_7]^-$ complex is only 4.3% at pH 8.7. At this pH the $[\text{Tb}_2(\text{BTP})_2(\text{OH})_7]^{2-}$ species is formed and it prevails at higher pH.

The species distribution diagrams with the plot of observed rate constant for the catalytic reaction make it possible to identify the reactive species present in those systems. For Sm^{3+} , Tb^{3+} and Er^{3+} we can see that the $\text{pH}-k_{\text{obs}}$ profiles are closely related to the distribution curves for the hydroxo complexes.

The equilibrium results were used to interpret the kinetics of the BDNPP hydrolysis reaction, where k_{obs} is the sum of the specific catalytic constants of each species present in solution:

$$k_{\text{obs}}[\text{Ln}_2(\text{BTP})_2]_{\text{total}} = k_1[\text{Ln}_2(\text{BTP})_2(\text{OH})_5]^+ + k_2[\text{Ln}_2(\text{BTP})_2(\text{OH})_6] + k_3[\text{Ln}_2(\text{BTP})_2(\text{OH})_7]^- + k_4[\text{Ln}_2(\text{BTP})_2(\text{OH})_8]^{2-} \quad (1)$$

Hydroxo complexes concentrations used in Eqn (1) were calculated from the total metal concentrations at each pH value. The specific rate constants of each species (k_1 – k_4) are shown in Table 3.

From the specific rate constants of each species, it is possible to make comparisons of the catalytic effects of all species, which have direct implications for the proposed reaction mechanism. However, the specific rate constants did not show significant variation for the different dinuclear hydroxo species present in solution. These results suggest that all dinuclear species are active species, starting from the pentahydroxide species.

Table 3. Rate constants for BDNPP hydrolysis by different Ln^{3+} complexes with BTP at 25 °C

	$10^2 k \text{ (s}^{-1}\text{)}$			
	Sm^{3+}	Tb^{3+}	Er^{3+}	La^{3+}
k_1	2.7	2.3	—	2.7
k_2	—	2.7	2.9	—
k_3	3.1	2.8	—	—
k_4	2.9	2.8	3.1	—

The simple observation that the dinuclear hydroxo complexes of Ln^{3+} are the active species in the catalysis of the BDNPP hydrolysis reaction is interesting from the point of view of the bimetallic cooperativity presented by some natural metalloenzymes.^{1,21} The participation of dinuclear complexes in phosphate ester hydrolysis reactions was proposed by Yatsimirsky and co-workers⁷ and allows one of the lanthanides to promote the binding act efficiently, while the second metal ion through association with a nucleophilic species induces a synchronic effect on the hydrolysis reaction rate. The overall effect results in both the ability of the metal ion to polarize P—O bonds and the placement of a nucleophilic species in a suitable position, an effect which results in charge, high coordination numbers and binding directionality.

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