

# Comparison of the Metal-Binding Properties of Nitrilotri(methylenephosphonic) Acid and Nitrilotriacetic Acid: Calcium(II), Nickel(II), Iron(III), and Thorium(IV) Complexes

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**Stability constants for Ca(II), Ni(II), Fe(III), and Th(IV) complexes of nitrilotri(methylenephosphonic) acid (NTMP) and nitrilotriacetic acid (NTA) were determined by a pH titration method. These stabilities were compared with those for the analogous complexes of nitrilodiaceticmethylenephosphonic acid and nitriloaceticdi(methylenephosphonic) acid. Replacement of carboxylate groups by phosphate groups, going from NTA to NTMP, resulted in a slight decrease in complex stability for Ca(II) and Ni(II) complexes. The Fe(III) complex of NTMP showed increased stability over the analogous NTA complex. Th(IV), however, formed complexes of about the same stability with NTA and NTMP. Both Fe(III) and Th(IV) formed insoluble complexes with NTMP below pH 5. Suggested analytical applications of phosphonic acid analogs are also reported.**

MOEDRITZER AND IRANI (1) have recently published a method for the direct synthesis of  $\alpha$ -aminomethylphosphonic acids in high yields. The ready availability and potential analytical usefulness of this new class of ligands have prompted an investigation of one of these compounds, nitrilotri(methylenephosphonic) acid (NTMP), and a comparison of its metal-binding properties with those of its well known analog, nitrilotriacetic acid (NTA). In 1949, Schwarzenbach *et al.* (2) demonstrated the high affinity of nitrilodiaceticmethylenephosphonic acid (NDAMP) for the Ca(II) ion. Assuming that substitution of additional phosphonate groups for carboxylate groups might further enhance the metal-binding abilities of these ligands, Westerbach *et al.* (3) studied the metal-binding properties of nitriloaceticdi(methylenephosphonic) acid (NADMP). They found that the NADMP-Ca(II) complex was slightly less stable than the NDAMP or NTA complexes. They ascribed this to the repulsion of the more negatively charged phosphonate groups. They suggested that these phosphonate ligands might show enhanced affinities for metal ions of +4 charge.

This paper describes the metal-binding properties of NTMP with Ca(II), Ni(II), Fe(III), and Th(IV), and compares these properties with those of the analogous ligands, NTA, NDAMP, and NADMP.

## EXPERIMENTAL

**Reagents.** Stock 0.2M Ca(II), Ni(II), Fe(III), and Th(IV) solutions were prepared from the analytical grade nitrates (or chloride in the case of Ca(II)) and standardized by the EDTA titration procedures of Schwarzenbach (4). Tetra-

propylammonium hydroxide (10% in water) and tetramethylammonium chloride were obtained from Eastman Organic Chemicals.

**Ligands.** Nitrilotri(methylenephosphonic) acid was synthesized according to the procedure of Moedritzer and Irani (1) and recrystallized two times; mp 210°-15° C, mol wt (titration) 302 (calcd for C<sub>3</sub>H<sub>12</sub>NO<sub>9</sub>P<sub>3</sub>: mol wt 299). Seed crystals, obtained from K. Moedritzer, were required for the initial crystallization. Nitrilotriacetic acid was obtained from Eastman Organic Chemicals.

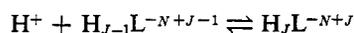
**pH Titrations.** pH titration curves were obtained with a Radiometer pH titrator using glass and calomel electrodes. The temperature of the titration vessel was maintained at 20° C, and nitrogen gas was bubbled through the solution to prevent CO<sub>2</sub> absorption and air oxidation. The pH meter was calibrated with NBS primary standard buffers (5).

A measured amount of ligand (7.9-15  $\mu$ mole) was pipetted into the vessel, followed by 4 ml of 0.25M tetramethylammonium chloride. A measured amount of metal ion was added and the final volume adjusted to 10 ml. Titration was begun with 0.1M tetrapropylammonium hydroxide as the titrant. Titration curves were automatically recorded at the rate of 0.5 pH unit/minute and were also checked manually for completeness of equilibration. The NTA-Th(IV) and NTMP-Fe(III) titrations were recorded at 0.06 pH unit/minute to ensure complete equilibration. The NTMP-Th(IV) titration was extremely slow in equilibrating. For this titration 10-ml samples corresponding to points on the titration curve were prepared in sealed vials and equilibrated for several days at 20° C, after which time the pH of each was measured.

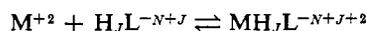
The pH meter readings were converted from activities to concentrations by the relationship,  $[H^+] = a_{H^+}/\gamma_{H^+}$ , assuming an activity coefficient  $\gamma_{H^+} = 0.799$  for the hydrogen ion at 20° C and 0.1 ionic strength (6).

## CALCULATIONS

The following equilibria were considered for the calculation of stability constants for the ligand, H<sub>N</sub>L, and metal ion, M<sup>+2</sup>.



$$K_{1j} = \frac{[H_jL^{-N+j}]}{[H^+][H_{j-1}L^{-N+j-1}]} \quad (1)$$



$$K_{MH_jL} = \frac{[MH_jL^{-N+j+2}]}{[M^{+2}][H_jL^{-N+j}]} \quad (2)$$

$$J = 0, 1, 2, \dots, N$$



- (1) K. Moedritzer and R. R. Irani, *J. Org. Chem.*, **31**, 1603 (1966).
- (2) G. Schwarzenbach, H. Ackerman, and P. Ruchstuhl, *Helv. Chim. Acta*, **32**, 1175 (1949).
- (3) S. Westerbach, K. S. Rajan, and A. E. Martell, *J. Am. Chem. Soc.*, **87**, 2567 (1965).
- (4) G. Schwarzenbach, "Complexometric Titrations," Interscience, New York, 1956, p. 60.

- (5) R. G. Bates, "Determination of pH, Theory and Practice," Wiley, New York, 1964, p. 75.
- (6) G. Kortüm and J. O'M. Bockris, "Textbook of Electrochemistry," Vol. II, Elsevier, New York, 1951, p. 672.

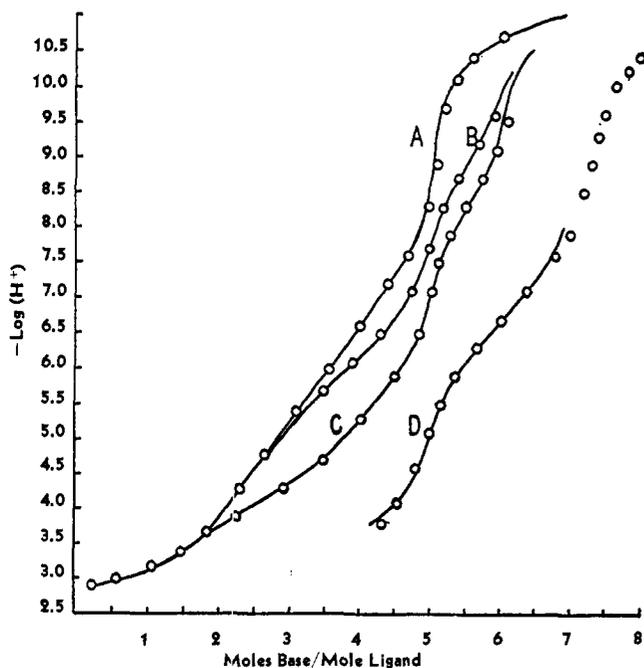


Figure 1. Titration curves for NTMP

A, Free ligand; B, Ca(II) (1:1); C, Ni(II) (1:1); D, Fe(III) (1:1) ( $CL = 7.9 \times 10^{-4}M$ )

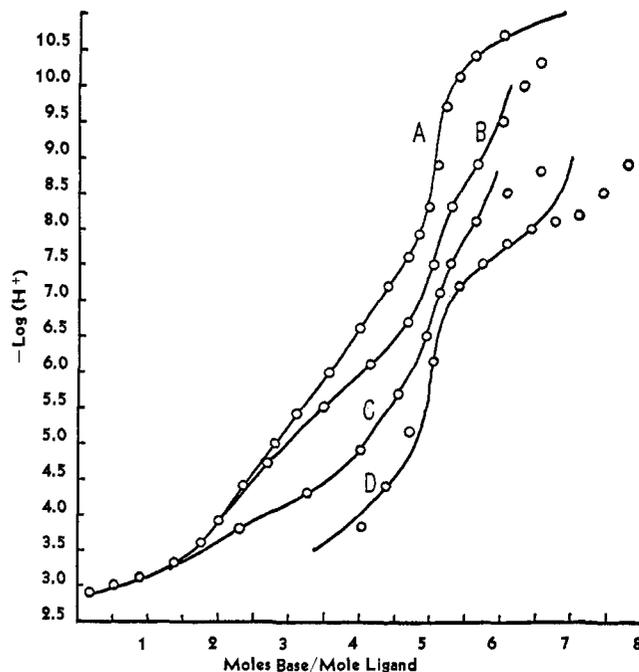


Figure 2. Titration curves for NTMP

A, Free ligand; B, Ca(II) (2:1); C, Ni(II) (2:1); D, Th(IV) (1:1) ( $CL = 7.9 \times 10^{-4}M$ )

Equations describing the total metal ion concentration ( $CM$ ), total ligand concentration ( $CL$ ), and electroneutrality can be written as follows, where  $BA$  = equivalents base added/mole ligand.

$$CM = [M^{+2}] + \sum_{J=0}^{J=N} [MH_J L^{-N+J+2}] \quad (4)$$

$$CL = \sum_{J=0}^{J=N} [H_J L^{-N+J}] + \sum_{J=0}^{J=N} [MH_J L^{-N+J+2}] \quad (5)$$

$$[H^+] + BA \cdot CL + 2[M^{+2}] = 2CM + [OH^-] + \sum_{J=0}^{J=N} \{(N-J)[H_J L^{-N+J}] + (N-J-2)[MH_J L^{-N+J+2}]\} \quad (6)$$

Simultaneous linear equations can be derived, from Equations 1-3, 5, and 6, in the form

$$\sum_{J=0}^{J=N} (N-J-XA)PK_J[H^+]^J [M^{+2}]K_{MH_JL} = \sum_{J=0}^{J=N} (XA+J-N)PK_J[H^+]^J \quad (7)$$

where  $PK_J = \prod_{i=0}^{i=J} K_{1i}$  (8),  $K_{10} = 1$ ,  $K_{11} = K_1$

$$XA = BA + \frac{[H^+]}{CL} - \frac{K_w}{[H^+]CL}$$

The following equation for free metal ion concentration can be derived from Equations 4 and 5.

$$[M^{+2}] = \frac{-B + \sqrt{B^2 + 4AC}}{2A} \quad (9)$$

where

$$A = \sum_{J=0}^{J=N} PK_J[H^+]^J K_{MH_JL}$$

$$B = (CL - CM)A + \sum_{J=0}^{J=N} PK_J[H^+]^J$$

$$C = CM \sum_{J=0}^{J=N} PK_J[H^+]^J$$

Acid association constants,  $K_{1J}$ , can be calculated from  $N$  linear equations (Equation 7 where  $[M^{+2}] = 0$ ) and Equation 8, using  $N$  sets of data ( $BA$  and  $[H^+]$ ) from the buffering regions of the titration curve.

First approximations of the stability constants,  $K_{MH_JL}$ , can be calculated from  $N$  simultaneous equations (Equation 7) using  $N$  sets of data ( $[H^+]$ ,  $BA$ , and an approximate free metal ion concentration). Second approximations for free metal ion concentrations are obtained using Equation 9. By an iterative process, exact values of the stability constants can be calculated.

Theoretical titration curves can be computed from the calculated acid association constants and stability constants using the relationship

$$BA = S4/S5 \quad (10)$$

where

$$S4 = \sum_{J=0}^{J=N} (N-J-D)PK_J(1 + K_{MH_JL}[M^{+2}])[H^+]^J$$

$$S5 = \sum_{J=0}^{J=N} PK_J(1 + K_{MH_JL}[M^{+2}])[H^+]^J$$

$$D = \frac{[H^+]}{CL} - \frac{K_w}{[H^+]CL}$$

The calculated constants can then be refined so that the best possible fit is obtained between the theoretical and experimental curves.

Equations 7, 9, and 10 are exactly the same for metal ions of charge other than +2. All of these calculations were pro-

Table I. Equilibrium Constants for NTMP<sup>a</sup>

Cation (metal:ligand)	Log $K_{ML}$				
	Log $K_{ML}$	Log $K_{MHL}$	Log $K_{MH_2L}$	Log $K_{MLOH}$	
Ca(II) (1:1)	6.25	4.15	2.7	...	
Ca(II) (2:1)	6.5	4.3	3.0	...	
Ni(II) (1:1)	9.3	6.7	5.1	...	
Ni(II) (2:1)	9.85	6.9	5.2	...	
Fe(III) (1:1)	14.6	9.9	6.0	-7.20	
Th(IV) (1:1)	12.6	9.3	6.2	-7.85	
Log $K_1$	10.9	Log $K_{12}$	7.35	Log $K_{13}$	5.92
		Log $K_{14}$	4.60	Log $K_{15}$	2.0
				Log $K_{16}$	1.9

<sup>a</sup> T = 20° C,  $\mu$  = 0.1 (Tetramethylammonium chloride).

grammed for an IBM 1620 computer. A paper describing in more detail the derivation of these equations and the computer programs is in preparation.

## RESULTS AND DISCUSSION

The pH titration curves for free and metal-complexed NTMP are shown in Figures 1 and 2. The solid lines represent theoretical titration curves computed for the constants shown in Table I, and the individual points represent experimental data. The acid association constants and stability constants for NTMP are given in Table I. The stability constants,  $K_{ML}$ , for NTMP-Ca(II) and NTMP-Ni(II) at the 2:1 metal-ligand ratio are greater than those calculated at the 1:1 ratio. These greater apparent constants probably reflect a small amount of metal hydrolysis near the sixth equivalence point, as is evident from the deviations of experimental points from the theoretical curves in Figure 2. Therefore, the values of  $K_{ML}$  calculated at the 1:1 ratios are probably more valid.

The Fe(III) and Th(IV) complexes of NTA, upon further titration, undergo hydrolysis and then ololation to form binuclear complexes such as (MLOH)<sub>2</sub>, and other more complex forms (7, 8). This is suggested by the noninteger inflection points in the titration curves for these complexes. The same sort of hydrolysis and ololation processes seems to be taking place for the Fe(III) and Th(IV) complexes of NTMP (see Figures 1 and 2). In order to obtain more valid values of  $K_{ML}$  for these complexes, an estimate of the equilibrium constant,  $K_{MLOH}$ , for the hydrolysis reaction,  $ML + H_2O \rightleftharpoons MLOH + H^+$ , was considered in the calculations of these theoretical curves.

Addition of Fe(III) or Th(IV) ions to a solution of the acidic NTMP (H<sub>6</sub>L) (pH 2.5) results in the formation of a colloidal precipitate which slowly dissolves above pH 4. Addition of these ions to NTMP above pH 5 results in no precipitation. Preliminary studies indicate complete precipitation of these ions, from a 0.01M acidic solution, in the form of 1:1 metal-ligand complexes. Zr(IV) and Hg(II) ions are also precipitated by NTMP under these conditions. Cu(II), Fe(II), and Ag(I) are not precipitated by NTMP.

Table II shows a comparison of the metal-binding properties of NTMP with those of analogous compounds where one or more phosphonate groups are replaced by carboxylate groups. The NTMP-Ca(II) complex is less stable than the analogous

Table II. Comparison of Metal Chelate Stabilities

Ligand	Log $K_{ML}$			
	Ca(II)	Ni(II)	Fe(III)	Th(IV)
NTA	6.8 <sup>a</sup>	10.3 <sup>a</sup>	11.8 <sup>a</sup>	12.0 <sup>a</sup>
	6.56 <sup>b</sup>	11.2 <sup>c</sup>	8.2 <sup>d</sup>	12.4 <sup>f</sup>
			15.87 <sup>e</sup>	
NDAMP	7.18 <sup>g</sup>	...	...	...
NADMP	6.2 <sup>h</sup>	...	14.65 <sup>h</sup>	...
NTMP	6.25 <sup>a</sup>	9.3 <sup>a</sup>	14.6 <sup>a</sup>	12.6 <sup>a</sup>

<sup>a</sup> T = 20° C,  $\mu$  = 0.1 (TMAcI) (This investigation).

<sup>b</sup> T = 20° C,  $\mu$  = 0.1 (KNO<sub>3</sub>) (9).

<sup>c</sup> T = 20° C,  $\mu$  = 0.1 (KCl) (10).

<sup>d</sup> T = 20° C,  $\mu$  = 0.1 (KNO<sub>3</sub>) (8).

<sup>e</sup> T = 20° C,  $\mu$  = 0.1 (KCl) (11).

<sup>f</sup> T = 25° C,  $\mu$  = 0.1 (KNO<sub>3</sub>) (8).

<sup>g</sup> T = 20° C,  $\mu$  = 0.1 (KCl) (2).

<sup>h</sup> T = 25° C,  $\mu$  = 0.1 (KNO<sub>3</sub>) (3).

NTA and NDAMP complexes. This is in accord with the NADMP-Ca(II) complex stability reported by Westerbach *et al.* (3). They ascribed this decrease in stability to repulsion of the more negatively charged phosphonate groups around the chelated metal ion. The decreased stability of the NTMP-Ni(II) complex as compared with its NTA analog also supports this assumption. The greater stabilities of the NTMP-Fe(III) and NADMP-Fe(III) complexes over the NTA-Fe(III) complex suggests that the greater positive charge on the central metal ion significantly reduces the repulsion of negative phosphonate groups. Using this analogy, one would expect an even greater increase in stability going from the NTA-Th(IV) complex to the NTMP-Th(IV) complex. No significant increase in stability was found, and these two complexes seem to have about the same stability. The reason for this is not clear. An investigation of additional complexes of NTMP and metal ions of +4 and greater charge should prove interesting in this respect.

While there is no apparent advantage in the use of NTMP as a complexone in complexometric titrations, this work does suggest the use of phosphonic acid analogs in other analytical applications. For example, the phosphonic acid analog of the Dowex A-1 chelating resin (which contains iminodiacetic acid groups) may be advantageous over the latter in terms of greater selectivity, at least between di- and trivalent metal ions. Such a resin could be easily synthesized by the method of Moedritzer and Irani (1). NTMP may also be useful as an analytical precipitating reagent for Fe(III) and Th(IV) ions.

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(7) R. L. Gustafson and A. E. Martell, *J. Phys. Chem.*, **67**, 576 (1963).

(8) R. C. Courtney, R. L. Gustafson, S. Chaberek, Jr., and A. E. Martell, *J. Am. Chem. Soc.*, **80**, 2121 (1958).

(9) T. Moeller and R. Ferrus, *Inorg. Chem.*, **1**, 55 (1962).

(10) G. Schwarzenbach and E. Freitag, *Helv. Chim. Acta*, **34**, 1492 (1951).

(11) *Ibid.*, p. 34, 1889.