Inorg. Chem. **2003**, *42*, 1994–2003

Inorganic Chemistry
Article

# Coordination Properties of Tris(2-carboxyethyl)phosphine, a Newly Introduced Thiol Reductant, and Its Oxide

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Received August 22, 2002

Acid–base properties and metal-binding abilities of tris(2-carboxyethyl)phosphine (TCEP), a newly introduced thiol group protectant, were studied in solution, using potentiometry, $^1$H and $^{31}$P NMR, and UV–vis spectroscopy, and also in the solid state by X-ray diffraction. Stability constants of complexes of the *P*-oxide of TCEP (TCEPO) were established by potentiometry. The list of metal ions studied included Ni(II), Cu(II), Zn(II), Cd(II), and Pb(II). Cu(II) catalyzed oxidation of TCEP to TCEPO. For all other systems ML complexes were found as major species at neutral pH with TCEP and TCEPO. Monoprotonated MHL species were also detected in weakly acidic conditions for all TCEP complexes and for the Pb(II) complex of TCEPO, while hydrolytic $MH_{-1}L$ complexes were found for TCEP at the weakly alkaline pH range. The $NiL_4$ complex was found to form at excess of TCEP. Overall, the complexes were found to be rather weak, with log $\beta_{ML}$ values around 3–5 for TCEP and 1.5–2.5 for TCEPO. The phosphorus p$K_a$ value for TCEP, 7.68, suggests that it can be a good buffer for studies at physiological pH.

## Introduction

Protection of thiol groups from oxidation is extremely important for the maintenance of activity and structure of many proteins. The most popular agents applied for this purpose are organic thiol compounds. Among these, dithiothreitol (DTT) is used most widely. This agent, introduced by Cleland, reduces linear disulfides quantitatively, forming a cyclic disulfide, *trans*-4,5-dihydroxy-1,2-dithiane.[1] Formation of a six-membered ring shifts the equilibrium constant of the thiol/disulfide reaction by 4 orders of magnitude, compared to monothiols, which makes DTT an effective thiol protectant. The use of DTT, as well as other thiol-based thiol protectants, has, however, limitations. These include poor reactivity at low pH, poor stability at high pH, and interference with thiol labeling and protecting groups and with spin labels.[2,3] We have recently published a study of coordination of several metal ions to DTT and demonstrated that it is a very strong chelating agent, and can interfere in chemical and biochemical studies of interactions of thiol compounds with essential and toxic metals.[4]

Trialkylphosphines, e.g., tributylphosphine, are very efficient and specific thiol reductants.[5] The major limitations of their application have been poor water solubility and foul odor. These disadvantages are not shared by odorless and water-soluble tris(2-carboxyethyl)phosphine (TCEP, CAS Registry Number 5961-85-3, called also, imprecisely, tricarboxyethylphosphine).[6] Despite that, this compound had been practically ignored for more than 20 years, until, following the publication of a convenient synthetic procedure,[7] TCEP (note that the same abbreviation can be found in the literature for several other compounds) has become available commercially. Since then it has gained increasing popularity, if at a rather slow pace. A Medline search performed in October 2002 indicated over 60 references to TCEP applied as thiol reductant, most of them after 1996.

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10.1021/ic025969y CCC: $25.00

TCEP was shown to reduce *trans*-4,5-dihydroxy-1,2-dithiane rapidly and completely at pH 5.[7] Subsequent studies demonstrated that TCEP is advantageous over DTT with respect to the pH range of application,[2] reactivity with various thiol labels, and resistance to oxidation by metal ions.[3] Coordination of TCEP to several metal ions, including Ni(II) and Zn(II), was studied previously in solution by potentiometry.[8] Also, crystal structures were obtained for TCEP complexes with Zn(II) and Co(II).[9,10] However, the data for toxicologically important Cd(II) and Pb(II) ions were not provided. The *P*-oxide of TCEP (TCEPO), the sole product of thiol reduction by TCEP, was first described in 1959, but its coordination properties were never studied.[11] In this paper we extend the previous studies and demonstrate that TCEP and TCEPO are much weaker chelators of biologically important metal ions than DTT, and therefore should be preferable in bioinorganic studies. The stabilities and spectroscopic parameters of complexes, given below, provide the basis for the planning and interpretation of such studies.

## Experimental Section

**Materials.** TCEP·HCl, phosphoric acid, TSP (sodium (3-trimethylsilyl)-2,2,3,3-tetradeuteriopropionate) were purchased from Sigma, and NaOH, perchlorates of Na(I), Cu(II), and Ni(II), nitrates of Cd(II) and Pb(II), MeOH, dimethylglyoxime, and $KNO_3$ were purchased from Merck. ZnO was obtained from BDH Chemicals Ltd, $HClO_4$ (60%) was purchased from Hopkin & Williams Ltd, $D_2O$ (99.9%) was from Cambridge Isotope Laboratories, $H_2O_2$ (30%) from Fluka, and $NH_3$ aq from POCH. $Zn(ClO_4)_2\cdot 6H_2O$ was prepared by reacting ZnO with $HClO_4$, recrystallizing the product from water, and drying crystals over $P_2O_5$. TCEPO was synthesized by adding 30% $H_2O_2$ (0.4 mmol) to a water solution of TCEP·HCl (0.2 mmol in 2 mL) and evaporation of the reaction mixture in vacuo. All volatile reagents evaporated, yielding the pure product as a hygroscopic white solid. This was dissolved in methanol, dried in vacuo, and stored over $P_2O_5$. The identity of the product was confirmed by mass spectrometry (*m/z*: theoretical 267.2, experimental 267.1), and its purity was checked by elemental analysis (% $C_{theor}$ 40.61, $C_{exp}$ 40.75(3); % $H_{theor}$ 5.68, $H_{exp}$ 5.4(1)) and by potentiometry as exceeding 99%. The red $Na_{10}[NiL_4]$ complex of TCEP was precipitated by adding ethanol to a water solution (40 mM $Ni(ClO_4)_2$ and 160 mM TCEP at pH = 9.0, controlled by NaOH). The precipitate formed was separated by centrifugation and filtration, washed with ethanol/water = 9/1 and ethanol, dried in vacuo, and stored over $P_2O_5$. The carbon and hydrogen contents of the solid state complex $Na_{10}[Ni(TCEP)_4]$ were measured using a Perkin-Elmer 2400 CHN analyzer. (% $C_{theor}$ 33.85, $C_{exp}$ 32.9(3); % $H_{theor}$ 3.79, $H_{exp}$ 3.85(4)). Nickel contents of the $Na_{10}[Ni(TCEP)_4]$ complex was measured gravimetrically, using dimethylglyoxime precipitation of the samples mineralized with nitric acid and neutralized with ammonia (% $Ni_{theor}$ 4.60, $Ni_{exp}$, 4.24(12)).

**Potentiometry.** Potentiometric titrations of TCEP, TCEPO, and their metal ion complexes in the presence of 0.1 M $KNO_3$ were performed at 25 °C using pH-metric titrations over the pH range 2.5−8.5 (Molspin automatic titrator) with 0.1 mmol dm$^{-3}$ NaOH as titrant. Changes in pH were monitored with a combined glass−Ag/AgCl electrode (Russell) calibrated daily in hydrogen ion concentrations by $HNO_3$ titrations.[12] Sample volumes of 1.5 cm$^{-3}$, TCEP and TCEPO concentrations of 2 mmol dm$^{-3}$, and M:L molar ratios of 1:0 (ligand titrations), 1:1, 1:2, and 1:4 were used. These data were analyzed using the SUPERQUAD program.[13] Standard deviations computed by SUPERQUAD refer to random errors only.

**Electronic Absorption (UV−Vis).** The electronic absorption spectra of TCEP and its complexes in solution were recorded at 25 °C on a Beckman DU-650 or on a Cary 50 Bio spectrophotometer over the spectral range 200−1100 nm, in 1 cm cuvettes. 0.187 and 1.87 mmol dm$^{-3}$ samples were used in free TCEP experiments, 1 mmol dm$^{-3}$ samples with M:L molar ratios of 1:1 were used for all metal ions studied, and additionally a M:L molar ratio of 1:4 was used for Ni(II). Absorption spectra of the $Na_{10}[NiL_4]$ complex of TCEP in the solid phase were collected on a Cary 500 UV−vis−NIR spectrophotometer over the range 200−2700 nm.

**NMR Spectroscopy.** $^1H$ and $^{31}P$ NMR spectra of 10 mmol dm$^{-3}$ TCEP and TCEPO samples in $D_2O$, both metal free and containing varied amounts of Zn(II) (1:1 and 1:3) or Ni(II) (1:1), were recorded at 298 K, on a Bruker AMX-300 spectrometer, at 300 MHz for $^1H$ and at 121.5 MHz for $^{31}P$. TSP was used as internal $^1H$ standard, while 0.5 mol dm$^{-3}$ $H_3PO_4$ in sealed capillaries was used as external $^{31}P$ standard. The $^{31}P$ spectra were proton-decoupled, the $^1H$ spectra were not decoupled from $^{31}P$.

**X-ray Crystallography.** The crystals of TCEP·HCl were used as obtained from Sigma. Crystals of Zn(II) complex of TCEP were obtained by dissolving TCEP·HCl (0.2 mmol) and $Zn(ClO_4)_2\cdot 6H_2O$ (0.2 mmol) in 5 mL of $H_2O$/MeOH (1:3), adding 1.5 mmol of $NH_3$ aq, and leaving overnight at room temperature. White crystals were washed with $H_2O$/MeOH (1:3) and $H_2O$. Crystallization of the Cd(II) analogue was performed similarly, using the nitrate instead of perchlorate as metal salt.

Crystal and molecular structures of TCEP·HCl, $[NH_4][Zn(TCEP)]\cdot 4H_2O$, and $[NH_4][Cd(TCEP)]\cdot 6.5H_2O$ were determined using data collected at room temperature on a CAD4 diffractometer with graphite-monochromatized Cu Kα radiation. The intensity corrections were applied (DECAY program).[14] An empirical absorption correction was applied by the use of the $\psi$-scan method (EAC program).[14,15]

The observed reflections with $F \geq 0\sigma(F)$, 5117 for TCEP·HCl, 1249 for $[NH_4][Zn(TCEP)]\cdot 4H_2O$, and 1321 for $[NH_4][Cd(TCEP)]\cdot 6.5H_2O$, were used to solve the structures by direct methods and to refine them by full-matrix least-squares using $F^2$.[16,17]

In all calculations the hydrogen atoms were placed geometrically at idealized positions, and set as riding with fixed thermal parameters equal to 1.33 times the equivalent isotropic thermal parameter of the parent atom. Anisotropic thermal parameters were refined for all non-hydrogen atoms. The final refinement for TCEP·HCl converged to $R = 0.0363$ for 426 refined parameters and 4940

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**Table 1.** Protonation Constants for TCEP and TCEPO[a]

| | TCEP | | TCEPO | |
|---|---|---|---|---|
| species | log $\beta$ | p$K_a$[c] | log $\beta$ | p$K_a$ |
| HL | 7.681(2)[b] | 7.681, 7.8(1),[d] 7.6(1)[e] | 4.857(4) | 4.857 |
| $H_2L$ | 11.987(3) | 4.306, 4.29(7)[d] | 9.073(4) | 4.216 |
| $H_3L$ | 15.578(3) | 3.591 | 12.622(5) | 3.549 |
| $H_4L$ | 18.445(4) | 2.867 | | |

[a] Constants obtained from potentiometric titrations at $I = 0.1$ ($KNO_3$) and 298 K, unless marked otherwise. [b] Standard deviations of the last digits are given in parentheses, at the values obtained directly from experiment. [c] Literature p$K_a$ values, obtained with a $NaClO_4$ as background electrolyte, were as follows: HL, 7.66; $H_2L$, 4.36; $H_3L$, 3.67; $H_4L$, 2.99. (Reference 8.) [d] Deuterium constants from $^{31}P$ NMR. [e] From UV−vis spectroscopy.

reflections with F ≥ 4σ(F). In calculations of Zn(II) and Cd(II) complexes, C−H, O−H, and N−H distances were set to 0.97, 0.93, and 0.90 Å, respectively. Directions of O−H and N−H were chosen in a way which yielded the shortest and sensible hydrogen bonds. For Zn(II) and Cd(II) the final refinements converged, respectively, to $R = 0.0287$ and 0.0688 for 168 and 193 refined parameters and 1239 and 1309 observed reflections with $F \geq 4\sigma(F)$, with inclusion of the extinction parameter into refinement (the obtained values of extinction parameter were 0.0047(5) and 0.0044(5), respectively). Data corrections were carried out with the Enraf-Nonius SDP crystallographic computing package;[14] structure solution was carried out with SHELXS[16] and structure refinement with SHELXL.[17]

The authors have deposited all crystallographic data for these structures with the Cambridge Crystallographic Data Centre.[18]

**Theoretical Calculations.** Structural calculations based on potential energy minimizations were done for TCEP complexes with Zn(II) and Cd(II), using the semiempirical MNDO/d method implemented under HyperChem 6.03. The following optimization criteria were used: RMS gradient 0.1 kcal/mol, convergence limit $< 10^{-8}$, and polarizability field strength $< 10^{-4}$ au.

## Results

**Solution Studies.** The molecule of TCEP contains four protonating groups: three carboxylates and the substituted phosphine. The protonation constants of TCEP and TCEPO, measured by potentiometry, are presented in Table 1. The assignment of the constants to the carboxylate and phosphine functions of TCEP was aided by a $^1H$ and $^{31}P$ NMR monitored pH* titration. Figure 1 presents the selected proton-decoupled $^{31}P$ spectra (A), the resulting titration curves and schematic structures of relevant protonation (deuteration) species (B). The TCEP molecule was found to be in the fast-exchange regime with regard to carboxyl group dissociations for both nuclei studied, as evidenced by the presence of a single spectroscopic species in the pH* range 2−7. The jump of $^{31}P$ chemical shift between pH* 3.5 and 5 was distinct enough to allow an accurate independent calculation of the p$K_a$ value for the $D_2L^- \rightarrow DL^{2-}$ reaction, which is provided in Table 1 along with the constants derived by potentiometry in $H_2O$.

Due to a slow kinetics of dissociation of the P−D bond (present instead of P−H due to the isotopic exchange of the proton in $D_2O$ solution) and/or the large chemical shift difference between the associated and dissociated forms, two separate, sharp signals of $DL^{2-}$ and $L^{3-}$ species could be observed and integrated. This provided an independent measure for the corresponding p$K_a$ value (also given in Table 1). A 1:1:1 deuterium coupling pattern could be seen in the $^{31}P$ spectra of the $DL^{2-}$ species of TCEP, with $^1J_{P-D}$ of 78 Hz (Figure 1).

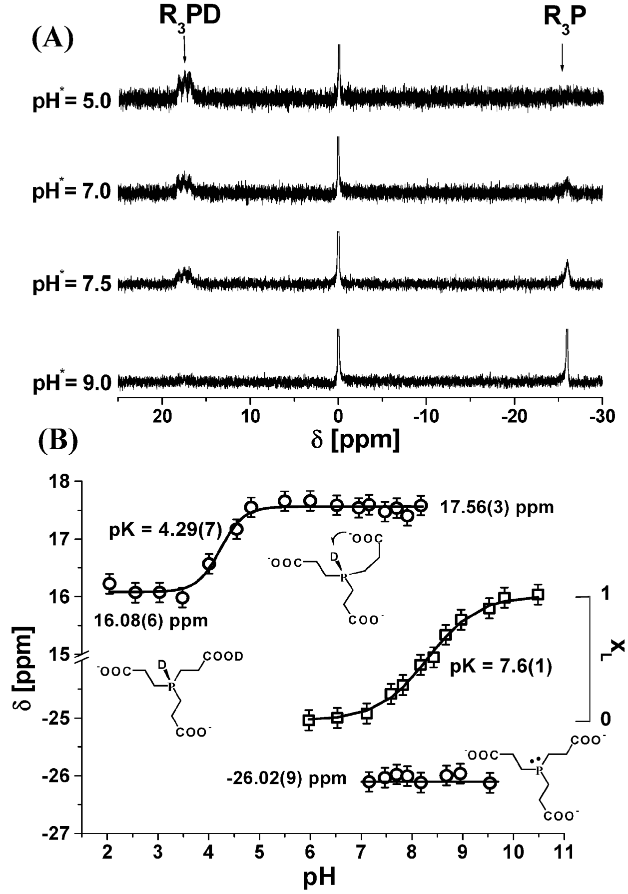

**Figure 1.** (A) $^{31}P$ NMR spectra of TCEP (10 mmol $dm^{-3}$) in $D_2O$ at 298 K. (B) $^{31}P$ NMR titration curves of TCEP and structural schemes of relevant protonation (deuteration) species, together with the calculated dissociation constants (numbers in parentheses denote standard deviations on the last digits). The left-hand-side curve is based on changes of $^{31}P$ chemical shift, while the right-hand-side curve is based on the normalized intensities of the $^{31}P$ signals of $DL^{2-}$ and $L^{3-}$ forms of TCEP. The arrow in the structure of the $DL^{2-}$ species denotes an interaction, described in the text.

**Table 2.** Chemical Shifts (δ) of Individual Protonation Species of TCEP and TCEPO

| | TCEP | | TCEPO | |
|---|---|---|---|---|
| species | $^1H$[a] | $^{31}P$[b] | $^1H$[a] | $^{31}P$[b] |
| $H_4L$ | 2.59, 2.32 | 16.1 | | |
| $H_3L$ | 2.59, 2.32 | 16.1 | 2.49, 2.05 | 56.9 |
| $H_2L$ | 2.59, 2.32 | 16.1 | 2.49, 2.05 | 56.9 |
| HL | 2.36, 2.19 | 17.6 | 2.30, 1.98 | 58.9 |
| L | 2.05, 1.49 | −26.0 | 2.30, 1.98 | 58.9 |

[a] δ (ppm, relative to TSP). [b] δ (ppm, relative to external 0.5 M $H_3PO_4$).

The $^1H$ and $^{31}P$ chemical shifts for $L^{3-}$ and $DL^{2-}$ species could be read directly from the spectra. For the remaining species they were calculated from the spectra for the pH* range 2−3.5, using appropriate p$K_a$ values, uncorrected for the isotope shift. These parameters are presented in Table 2. The signals of methylene protons in the $^1H$ spectra were multiplets, exhibiting proton−proton as well as phosphorus−proton coupling (no decoupling was applied).

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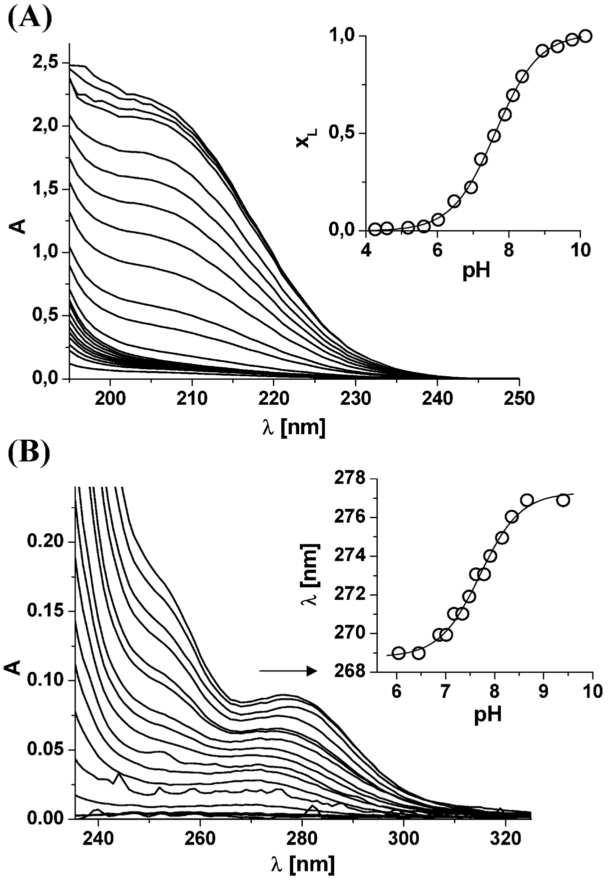

**Figure 2.** UV titrations of TCEP. (A) High-energy band at 205 nm. Inset presents the dependence of the band intensity on pH, converted to the molar fraction of $L^{3-}$ (○) and the fit to a p$K_a$ value of 7.62(2) (—). Spectra recorded at 298 K and $I = 0.1$ (NaClO$_4$), for [TCEP] of 0.187 mmol dm$^{-3}$. (B) Lower energy bands at 253 and 269−277 nm. Arrow indicates the direction of shift of the maximum. Inset presents the dependence of the low-energy band position on pH (○) and the fit to a p$K_a$ value of 7.70(5) (—). Spectra recorded at 298 K and $I = 0.1$ mol dm$^{-3}$ (NaClO$_4$), for [TCEP] of 1.87 mmol dm$^{-3}$.

TCEPO, possessing only carboxylate proton acceptors, exhibited the averaged spectrum in the whole pH* range studied. The chemical shifts for its individual forms, obtained from speciation-aided calculations, are also presented in Table 2.

Spectrophotometric titrations of TCEP revealed the presence of three absorption bands between 205 and 280 nm. All three bands gain intensity along with phosphine deprotonation, as presented in Figure 2. The parameters for the deprotonated species $L^{3-}$ are 205 nm (12000 mol$^{-1}$ cm$^{-1}$), 253 (shoulder, 95 mol$^{-1}$ cm$^{-1}$), and 270 (48 mol$^{-1}$ cm$^{-1}$).

Stability constants of TCEP and TCEPO complexes were calculated from potentiometric titrations. In all cases the [ML]$^-$ species were major complexes in equimolar solutions at neutral pH. The TCEP complexes of Cu(II) could not be studied due to immediate catalytic oxidation of TCEP by Cu(II) to TCEPO. In most cases, the titrations were terminated by visible precipitation of metal hydroxides in weakly alkaline solutions. As a result, the calculations were performed for the pH values below 8.5, with inclusion of hydrolytic forms; only for Ni(II) complexes of TCEP they were extended up to pH 9.5. The constants are given in Table 3, along with the comparable literature data.[8]

**Table 3.** Logarithmic Values of Stability Constants and Related Quantities for Complexes of TCEP and TCEPO[a]

| | Cu(II) | Cd(II) | Pb(II) | Ni(II) | Zn(II) |
|---|---|---|---|---|---|
| TCEP | | | | | |
| log $\beta$(MHL) | | 9.96(4)[b] | 10.05(1) | 9.49(6) | 9.00(9) |
| log $\beta$(ML) | | 5.00(1) | 4.208(4) | 4.173(8) | 2.91(1) |
| log $\beta$(MH$_{-1}$L)[c] | | −3.06(1) | −4.330(8) | | −5.92(9) |
| log $\beta$(ML$_4$) | | | | 12.31(5) | |
| log $\beta$(MHL) − log $\beta$(HL) | | 2.28 | 2.37 | 1.81 | 1.32 |
| log $\beta$(MHL) − log $\beta$(ML) | | 4.96 | 5.84 | 5.32 | 6.09 |
| log $K^C$ | | 4.54 | 3.76 | 3.71/4.28[d] | 2.47 |
| TCEPO | | | | | |
| log $\beta$(MHL) | | | 7.32(2) | | |
| log $\beta$(ML) = log $K^C$ | 2.46(1) | 1.75(1) | 2.63(1) | 1.32(3) | 1.50(2) |

[a] Constants obtained from potentiometric titrations at $I = 0.1$ (KNO$_3$) and 298 K. [b] Standard deviations of the last digits are given in parentheses, at the values obtained directly from experiment. [c] Tentative values due to coincident metal hydroxide formation. [d] The higher value is valid for 10-fold and higher excess of TCEP over Ni(II).

Spectrophotometric titrations were performed for 1:1 samples of TCEP with all metal ions. In addition, Ni(II) complexes were studied at a range of metal-to-ligand ratios, up to 1:4. Also a Ni(II) titration of TCEP at a constant pH of 8.5 was performed. The relevant speciation diagrams are presented in Figure 4. The relative intensities of characteristic absorption bands of complexes are overlaid on the potentiometric speciation plots. The UV−vis spectra of TCEP complexes are presented in Figure 5. Part A of this figure presents the CT region for [ML]$^-$ complexes, part B shows the spectrum of the [NiL$_4$]$^{10-}$ complex at pH 9.5, and part C presents the absorption spectrum of the red powder of Na$_{10}$[NiL$_4$]. The identity of the latter species was confirmed by ethanol precipitation and elemental analysis C, H, Ni). The parameters of UV−vis spectra of [ML]$^-$ complexes are as follows: Pb(II), 274 nm (359 mol$^{-1}$ cm$^{-1}$); Cd(II), 272 nm (107 mol$^{-1}$ cm$^{-1}$); Zn(II), 270 nm (273 mol$^{-1}$ cm$^{-1}$); Ni(II) 270 nm (365 mol$^{-1}$ cm$^{-1}$). For the [NiL$_4$]$^{10-}$ complex there are three characteristic bands, at 251 nm (5980 mol$^{-1}$ cm$^{-1}$), 275 nm (3050 mol$^{-1}$ cm$^{-1}$), and 437 nm (187 mol$^{-1}$ cm$^{-1}$).

Attempts to study complex formation by NMR failed, due to a partial hydrolysis and precipitation of metals at neutral pH, at the relatively high concentrations required in those experiments.

**Structural Studies.** Crystals of TCEP·HCl purchased from Sigma were of sufficient quality to be used directly in X-ray structure determination. TCEP·HCl crystallized in the orthorhombic system, in space group *Pca*2$_1$ with the unit cell consisting of 8 molecules.

Crystals of TCEP·HCl belong to a chiral space group, despite not being chiral themselves. The independent part of the unit cell (Figure 3) contains two phosphine cations and two chlorine anions. Despite the fact that the central phosphorus atom has a 3-fold symmetry axis, the whole molecule does not have this symmetry. Both molecules in the independent part have an approximate symmetry plane. Two 2-carboxyethyl moieties are bent toward each other and

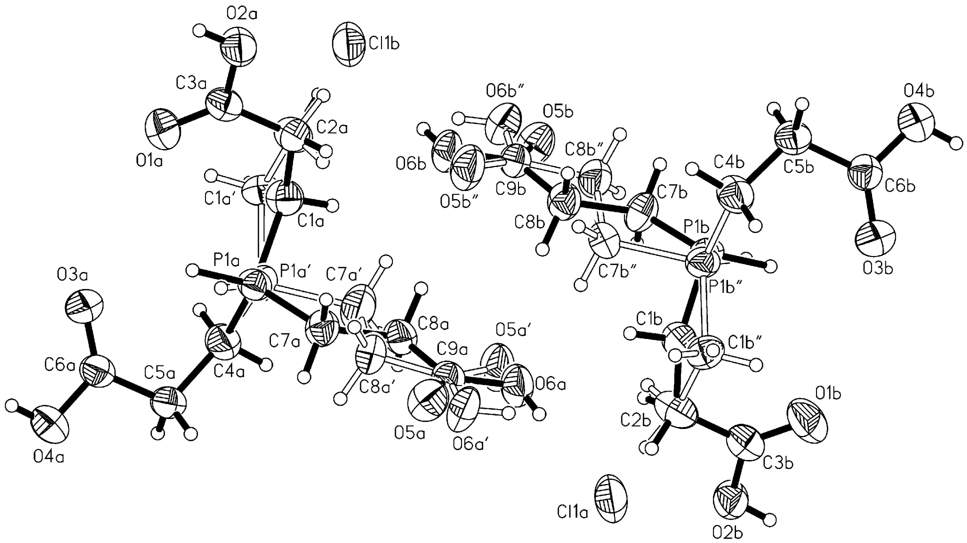

**Figure 3.** View of the two inequivalent molecules of TCEP, making up the independent part of the TCEP•HCl crystal structure, with ellipsoids at 50% probability. Clear bonds mark the alternative positions of the disordered ethylcarboxyl groups with the lower sof.

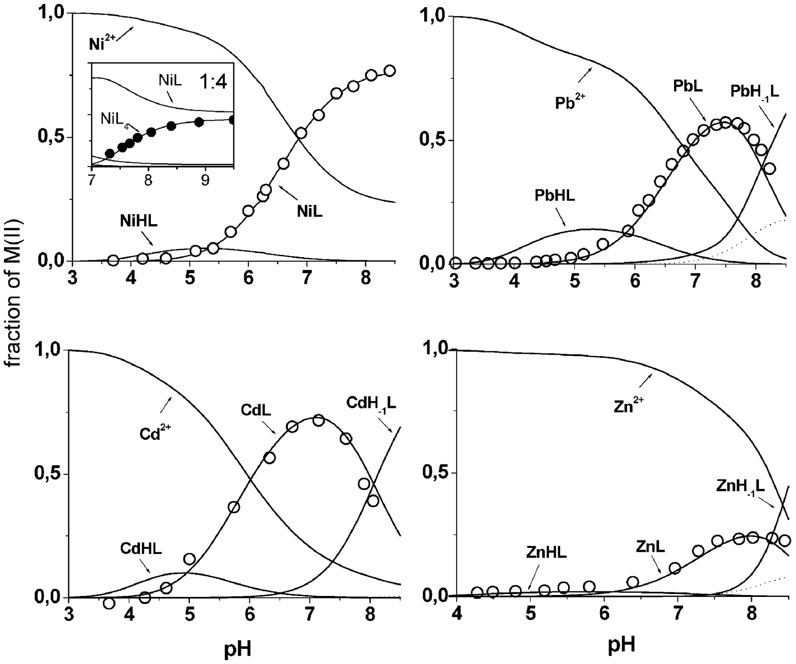

**Figure 4.** Speciation plots of TCEP complexes, calculated from potentiometric stability constants for $c_M = c_{TCEP} = 1$ mmol dm$^{-3}$, and additionally for $c_{TCEP} = 4$ mmol dm$^{-3}$ with Ni(II), as used for spectroscopic studes; (○) concentration-adjusted intensities of the ligand absorption band [Ni(II), $\lambda_{max} = 270$ nm; Pb(II), $\lambda_{max} = 273$ nm; Cd(II), $\lambda_{max} = 273$ nm; Zn(II), $\lambda_{max} = 271$ nm]; (●) Ni(II), $\lambda_{max} = 437$ nm; (···), hydroxo complexes.

lie on opposite sides of this plane of symmetry. The third one lies approximately on this plane. This group is structurally disordered. The disorder is observed in the occurrence of two alternative placements of the 2-carboxyethyl chain. The central carbon atom in the carboxyl group has the same position for both orientations, but the oxygen atoms are shifted. The occupancy factors for both positions of the two molecules are not equal. They are 0.29/0.71 and 0.48/0.52 for the first and second molecules, respectively. The chlorine anion is located between the carboxyl groups of symmetry-related molecules. The first chlorine anion is surrounded only by the symmetry-related first molecule, and the second anion only by the second molecule. The relevant interatomic distances are provided in Table 5.

The water-insoluble crystals of Zn(II) and Cd(II) complexes of TCEP were obtained from methanol−water solutions. Both complexes crystallized in the hexagonal system, in space group *P*6$_3$ with the unit cell consisting of four molecules. Figures 6 and 7 present the molecular structure and placement of molecules in their unit cells for Zn(II) and Cd(II) complexes, respectively.

The complex of TCEP with Zn(II) consists of a single molecule of TCEP, one $Zn^{2+}$ cation, and one $NH_4^+$ cation. There are two different $Zn^{2+}$ cations present in the independent part of the unit cell (Zn1a and Zn1b, as shown in Figure 6). Both of them have tetrahedral coordination spheres, consisting of three carboxyl oxygen atoms and one phosphorus atom. The independent part consists of two $Zn^{2+}$ ions

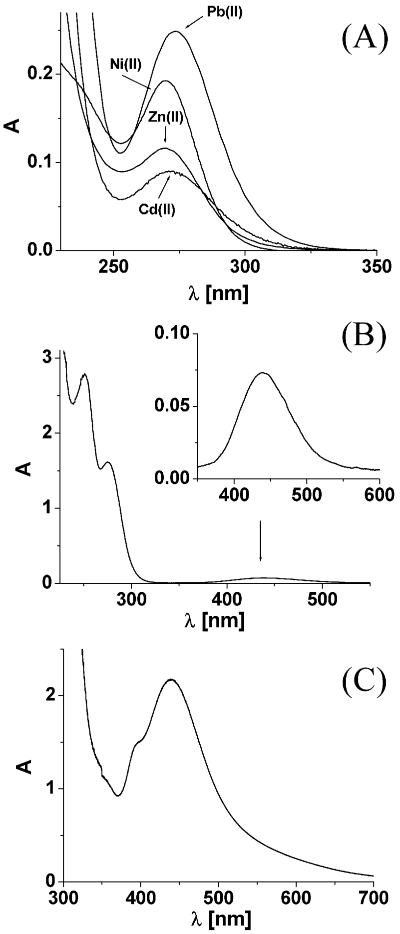

**Figure 5.** (A) The UV−vis absorption band near 270 nm for the ML complexes at the maximum of their formation in 1 mmol dm$^{-3}$solutions; Pb(II), pH 7.5; Cd(II), pH 7.2; Ni(II), pH 8.3; Zn(II), pH 8.0. (B) The UV−vis spectrum recorded for [TCEP] = 4 mmol dm$^{-3}$ and [Ni(II)] = 1 mmol dm$^{-3}$, at pH 0.5 (maximum of the formation of the $[NiL_4]^{10-}$ complex). (C) The absorption spectrum of the powder of $[Na_{10}NiL_4]$.

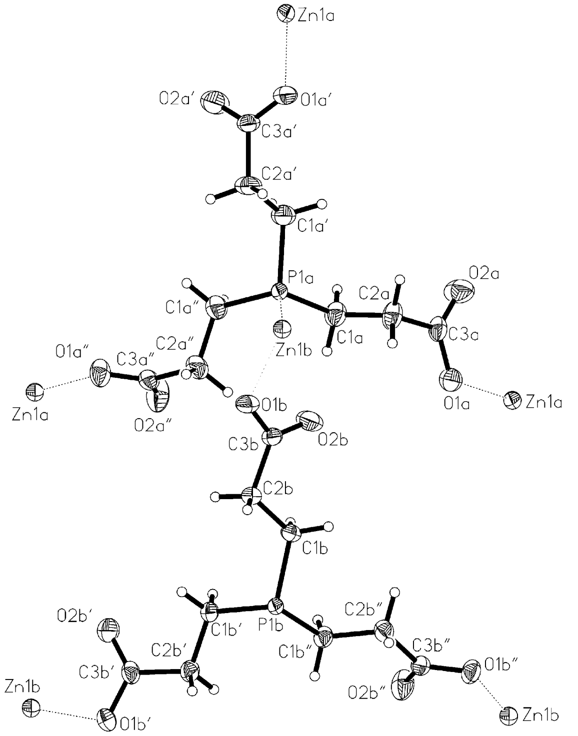

**Figure 6.** View of the independent part of the crystal structure of the $[NH_4][Zn(TCEP)]\cdot 4H_2O$ complex, omitting the water molecules and ammonium cations, with ellipsoids at 50%. The atoms of the calculated independent unit are numbered (the other two arms of TCEP are symmetry-related moieties). Open bonds connect the atoms with lower occupation factors in the disorder region.

and two phosphorus atoms, located in the special positions on the 3-fold axis of the polymeric structure. Each of 2-carboxyethyl moieties, coordinating a given $Zn^{2+}$ ion, is attached to a different phosphorus atom. The spatial network contains gaps, filled by water molecules and $NH_4^+$ cations. An $NH_4^+$ cation with the occupancy factor 0.67 occurs in the independent part. Its fulfiller is a water molecule O1 with occupancy factor 0.33. Additionally, there are three other water molecules present in the gaps, O2 and O3 with split position, and O4, which has a partial occupation factor. Because the occupancy factor of $Zn^{2+}$ and phosphorus is 0.67, the unit cell contains 4 molecules of TCEP, 4 $Zn^{2+}$ cations, 4 $NH_4^+$ cations, and 27 water molecules. The water molecules are loosely connected by hydrogen bonds to the spatial network. Due to this, the thermal factors are relatively high. The environments of the $Zn^{2+}$ cations are not identical. The first Zn atom has slightly shorter distances to the surrounding oxygen and phosphorus atoms than the second one. The relevant interatomic distances are provided in Table 6. The Cd(II) complex exhibits similar structural features. The differences are the following: the lengths of $Cd^{2+}$ bonds are slightly longer, due to its bigger ionic radius. This results in a larger volume inside the cluster formed by TCEP and $Cd^{2+}$, so that the number of water molecules is 6.5 per one TCEP molecule, in comparison to 4 in the Zn(II) case. A more relaxed structure allows for a partial disorder, exhibited by one of the two TCEP molecules in the independent part (Figure 7).

Crystallographic CIF files are provided in the Supporting Information section.

Molecular mechanics calculations were performed for the assumed $[ML]^-$ complexes of TCEP with Zn(II) and Cd(II). The resulting structure of the zinc complex is presented in Figure 8, the structure of the Cd(II) complex being very similar. The following relevant interatomic distances and angles were found: M−P, 2.305 Å (Zn), 2.481 Å Cd(II); M−O1,O3,O5, 1.955 Å (Zn), 2.132 Å (Cd); O1,O3,O5−M−P, 109.88° (Zn), 90.71° (Cd); O1,O3,O5−M−P−C3,C6,C9, 0.71° (Zn), 1.14° (Cd).

## Discussion

**Properties of Ligands.** The combined results of potentiometric and NMR titrations confirmed the assignments of three lower p$K_a$ values of TCEP to carboxyl functions, and of the highest one to the phosphine, thus providing the firm support for previous assignments.[8] The values obtained presently are also in good agreement with the previous ones (see Table 1). The lower acidicity of TCEPO carboxyls, by 0.6 log unit on average, is due to the absence of the positive charge on phosphorus. The average separation of carboxyl p$K_a$ values in TCEP (0.720 ± 0.005) is markedly higher from

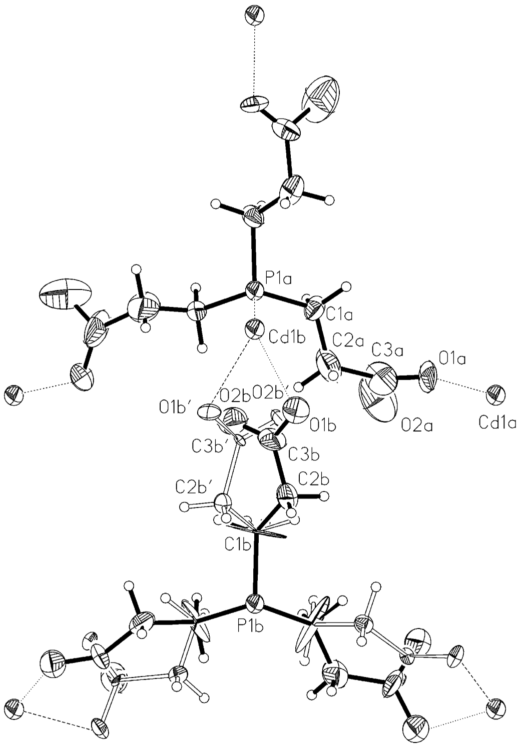

**Figure 7.** View of the independent part of the crystal structure of the $[NH_4][Cd(TCEP)]\cdot 6.5H_2O$ complex, omitting the water molecules and ammonium cations, with ellipsoids at 50%. The atoms of the calculated independent unit are numbered (the other two arms of TCEP are symmetry-related moieties). Open bonds connect the atoms with lower occupation factors in the disorder region.

**Table 4.** Crystal Data and X-ray Experimental Details

| parameter | TCEP·HCl | $[NH_4][Zn(TCEP)]\cdot 4H_2O$ | $[NH_4][Cd(TCEP)]\cdot 6.5H_2O$ |
|---|---|---|---|
| mol formula | $C_9H_{16}ClO_6P$ | $C_9H_{24}NO_{10}PZn$ | $C_9H_{29}NO_{10.5}PCd$ |
| fw | 286.64 | 402.63 | 494.70 |
| cryst syst | orthorhombic | hexagonal | hexagonal |
| space group | $Pca2_1$ | $P6_3$ | $P6_3$ |
| $a$ (Å) | 23.464(2) | 11.528(2) | 11.630(2) |
| $b$ (Å) | 6.7161(4) | 11.528(2) | 11.630(2) |
| $c$ (Å) | 15.9813(14) | 15.168(7) | 15.731(9) |
| α (deg) | 90.0 | 90.0 | 90.0 |
| $\beta$ (deg) | 90.0 | 90.0 | 90.0 |
| $\gamma$ (deg) | 90.0 | 120.00 | 120.00 |
| $V$ (Å$^3$) | 2518.4(4) | 1745.7(9) | 1842.5(12) |
| $Z$ | 8 | 4 | 4 |
| $D_c$ (g/cm$^3$) | 1.512 | 1.532 | 1.783 |
| $\mu$ (cm$^{-1}$) | 4.055 | 32.56 | 10.894 |
| cryst dimens (mm) | 0.15 × 0.25 × 0.3 | 0.25 × 0.25 × 0.3 | 0.25 × 0.25 × 0.45 |
| radiation, $\lambda$ (Å) | Cu Kα, 1.54178 | Cu Kα, 1.54184 | Cu Kα, 1.54184 |
| $R$ (obsd) | 0.0363 | 0.0287 | 0.0688 |
| $R_w$ (obsd) | 0.0996 | 0.0814 | 0.1977 |
| $S_{obs}$ | 1.060 | 1.180 | 1.085 |
| weighting coeff[a] | | | |
| $m$ | 0.0662 | 0.0510 | 0.114400 |
| $n$ | 0.5747 | 0.8318 | 15.21803 |
| extinction coeff.[b] | | | |
| $k$ | n/a | 0.0047(5) | 0.0042(5) |
| $T_{meas}$ | 293(2) | 293(2) | 293(2) |

[a] Weighting scheme $w = [\sigma^2(F_o^2) + (mP)^2 + nP]^{-1}$, where $P = (F_o^2 + 2F_c^2)/3$. [b] Extinction method SHELXL, extinction expression $F_c^* = kF_c[1 + 0.001F_c^2\lambda^3/\sin(2\theta)]^{-1/4}$.

**Table 5.** Bond Lengths (Å) for Non-Hydrogen Atoms in TCEP·HCl

| | | molecule a | | molecule b | |
|---|---|---|---|---|---|
| sof:[a] | | 0.71 | 0.29 | 0.52 | 0.48 |
| P1 | C1 | 1.794(9) | 1.77(2) | 1.807(12) | 1.738(12) |
| P1 | C4 | 1.844(4) | 1.653(10) | 1.850(9) | 1.800(10) |
| P1 | C7 | 1.776(5) | 1.834(14) | 1.751(9) | 1.863(11) |
| C1 | C2 | 1.496(9) | 1.64(2) | 1.549(10) | 1.565(11) |
| C2 | C3 | 1.496(4) | | 1.491(4) | |
| C3 | O1 | 1.203(3) | | 1.207(4) | |
| C3 | O2 | 1.313(3) | | 1.329(3) | |
| C4 | C5 | 1.522(4) | | 1.516(4) | |
| C5 | C6 | 1.494(4) | | 1.497(4) | |
| C6 | O3 | 1.197(3) | | 1.203(3) | |
| C6 | O4 | 1.333(3) | | 1.327(3) | |
| C7 | C8 | 1.521(5) | 1.51(2) | 1.517(6) | 1.512(8) |
| C8 | C9 | 1.506(5) | 1.472(9) | 1.485(6) | 1.496(6) |
| C9 | O5 | 1.210(4) | 1.245(9) | 1.243(5) | 1.248(6) |
| C9 | O6 | 1.312(4) | 1.318(10) | 1.307(5) | 1.295(5) |

[a] Structure occupancy factor.

**Table 6.** Bond Lengths (Å) for Non-Hydrogen Atoms in Zn(TCEP) and Cd(TCEP)

| | | molecule a | molecule b |
|---|---|---|---|
| P1 | Zn1[a] | 2.385(2) | 2.357(2) |
| P1 | C1 | 1.841(4) | 1.809(4) |
| C1 | C2 | 1.522(6) | 1.515(5) |
| C2 | C3 | 1.517(5) | 1.512(5) |
| C3 | O2 | 1.226(5) | 1.238(5) |
| C3 | O1 | 1.272(5) | 1.279(5) |
| O1 | Zn1 | 1.987(3) | 2.011(3) |
| P1 | Cd1[a] | 2.487(9) | 2.540(9) |
| P1 | C1 | 1.83(2) | 1.78(2) |
| C1 | C2 | 1.46(3) | 1.35(3) |
| C2 | C3 | 1.50(3) | 1.47(3) |
| C3 | O2 | 1.24(3) | 1.25(2) |
| C3 | O1 | 1.36(3) | 1.28(2) |
| O1 | Cd1 | 2.22(1) | 2.29(2) |

[a] P1a···M1b, P1b···M1a($1 - x$, $1 - y$, $0.5 + z$), M = Zn, Cd.

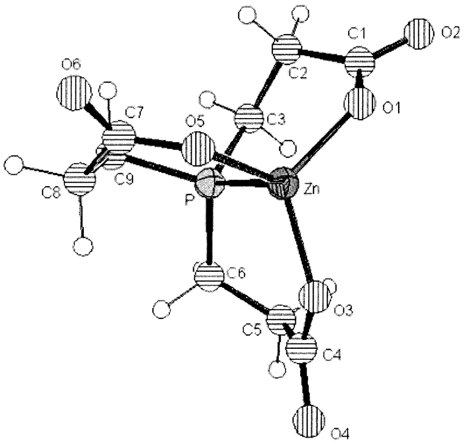

**Figure 8.** The solution structure of the Zn(II) complex of TCEP, obtained from molecular mechanics calculation using the MNDO/d method implemented under HyperChem 6.03 moieties.

the statistical value for three equivalent protonating groups (0.478), thus indicating the presence of O−H···O hydrogen bonds between carboxylic oxygens in $H_2L^-$ and $HL^{2-}$ species[19] ($H_nL^{n-3}$ designates TCEP or TCEPO at a given protonation state). A slightly lesser separation in TCEPO (0.654 ± 0.013) is likely due to small steric hindrance exerted by phosphorus-bound oxygen. The $^1H$ and $^{31}P$ chemical shifts of TCEP and TCEPO are typical for phosphines and their oxides.[20]

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The presence of a very strong P−D coupling in the $DL^{2-}$ species present in NMR samples at pH 4.5−7.5 is a rare, but not unprecedented, feature in phosphorus compounds. The $^1J_{PD}$ constant of 78 Hz can be recalibrated into a $^1J_{PH}$ constant of ca. 500, using a ratio of gyromagnetic coefficients of H and D of ca. 6.5. A $^1J_{PH}$ constant as high as 852 Hz was reported recently in 1-hydro-5-carbaphosphatrane.[21] The occurrence of the coupling indicates a very slow exchange of phosphorus-bonded $D^+$, likely due to its caging by carboxylate groups.

There are three bands present in the UV spectrum of TCEP. The most intense one, at 205 nm, has a position and intensity similar to that seen previously for simple trialkylphosphines in the gas phase.[22]

The X-ray study demonstrated the geometrical inequivalence of 2-ethylcarboxyl chains, indicated already by $^{31}P$ NMR studies. This is due to steric and electrostatic repulsion between these chains. Two of them are symmetrical, while the third is bent "up" toward the phosphorus atom. The deprotonation of the first two groups results in the increase of mutual repulsion, leading to a further deformation of the molecule, which is seen in the shift of position of the $^{31}P$ resonance by 2 ppm. However, this effect is not reflected in the spread of p$K_a$ values for carboxyl groups.

**Complex Formation.** The character of bonding in [MHL] complexes can be determined by comparing their stabilities with those of the corresponding $[ML]^-$ complexes of TCEPO, for which the three-carboxylate coordination is the sole option. The subtraction of phosphine p$K_a$ from log $\beta_{MHL}$ values (Table 3) yields quantities which can be used for direct comparisons. The TCEP complexes of Cd(II), Pb(II), and Zn(II) are slightly weaker than their TCEPO counterparts. In conjunction with the lower acidity of TCEPO carboxylic groups, this indicates the same coordination mode. Another piece of evidence is provided by high values of p$K_a$ for the ML complex formation, unfeasible for carboxylates. The 1:1 Ni(II) complex of TCEP is more stable than that of TCEPO (by 1 log unit). This is due to specific geometrical requirements of the octahedral high-spin Ni(II) ion, rather than the presence of a Ni−P bond. Also spectroscopy clearly points against the presence of a Ni−P bond in the [NiHL] complex (see below).

It follows that the stabilization of $[ML]^-$ compared to [MHL] is due to the formation of the metal−phosphorus bond. Its effectiveness, demonstrated by the lowering of the phosphine p$K_a$ vs the free TCEP value of 7.68 (Table 1), is particularly high for Cd(II) and the lowest for Zn(II). The formation of the P-bonded complex can be followed by UV spectra, in which a band at 270 nm gains intensity in a fashion similar to that seen for TCEP deprotonation, with only slight differences in $\lambda_{max}$ (cf. Figures 2 and 5). A similar band was assigned previously for square-planar complexes of Ni(II) with aliphatic phosphines, like $(CH_3)_3P$ as a LMCT transition.[23,24] It indeed accompanies the formation of an analogous TCEP complex $[NiL_4]^{10-}$, but is present, albeit much weaker, in the protonated TCEP, and is seen for the high-spin and low-spin Ni(II) complexes, for complexes of $d^{10}$ ions Zn(II) and Cd(II), and even for those of Pb(II), the main group ion. Therefore it is likely that there is an intraligand transition perturbed by complexation at this wavelength, coinciding with a CT transition.

The onset of hydroxide precipitation could be noticed at pH 8 for Cd(II), Pb(II), and Zn(II). Potentiometry detected also intermediate hydrolytic complexes of a general formula $[MH_{-1}L]^{2-}$ for these metals, which most likely possess hydroxide ions in their coordination spheres. These complexes are likely to be thermodynamically unstable intermediates of the polymeric hydroxide formation, and so their stability constants, provided in Table 3, are tentative.

Ni(II) is unique among the metal ions studied by its ability to form a $[NiL_4]^{10-}$ complex at TCEP excess. Such stoichiometry is common for aliphatic phosphines devoid of alternative donor groups.[23,24] These complexes are square-planar, as evidenced by, among other things, their absorption spectra.

The constants for $[ML]^-$ complexes of Zn(II) and Ni(II) can be compared with those obtained previously.[8] The Zn(II) constant is practically identical, log $\beta$ of 2.91 vs 2.92, while that for Ni(II) is higher in our studies, 4.27 vs 3.80. This deviation is likely due to an inappropriateness of the complexation model assumed by the previous authors (who missed the actual $[ML_4]^{10-}$ species). Also, the precipitation, which prevented them from studying Pb(II), did not occur in our studies, perhaps due to lower concentrations used in our studies, or a different background electrolyte (nitrate instead of perchlorate). Interestingly, the $[ML]^-$ complexes of TCEP are uniformly slightly stronger than those of phosphinotriethanoic acid (or tricarboxymethylphosphine, TCMP): by ca. 0.4 log unit for Ni(II), Zn(II), and Pb(II) and 1.5 log units for Cd(II).[25] The reasons for the size of these differences are likely to be composite: the acidity of TCMP phosphorus is higher than that of TCEP by 2.2 log units, but this is partially compensated by the more stable five-membered chelate rings formed with TCMP carboxyl oxygens, in contrast to six-membered ones for TCEP.

The solution studies, done at various concentrations, gave only evidence for the formation of monomeric TCEP complexes, of a kind represented in Figure 8, which was obtained by molecular mechanics calculations. On the other hand, the crystal structures of Zn(II) and Cd(II) complexes presented in this work, as well as the Zn(II) and Co(II) complexes, presented previously by Podlaha et al.,[9,10] contain a network of metal and phosphine molecules. Apart from the similarities, there are also some interesting differences between the comparable Zn(II) structures. In both, each metal ion is coordinated to donor groups from four different TCEP

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molecules and there are two inequivalent Zn(II) atoms and TCEP molecules in each structure. However, the previous structure[9] contained a pentagonal $[Zn(H_2O)_5]^{2+}$ cation, while ours contains a $NH_4^+$ cation surrounded by three water molecules. This difference dictates a change of space group, from $P6_5$ (or $P6_1$) to $P6_3$. This comparison indicates that the formation of spatial lattices dictates the overall structures of crystals of TCEP complexes, which are flexible enough to adopt various voluminous counterions and spatial lattice guests.

The polymeric solid state structures differ considerably from the monomeric ones, present in solution, but the sets of donor atoms around metal ions are the same in both cases. The crystals of Zn(II) and Cd(II) complexes, once precipitated with methanol, are water insoluble and nonhygroscopic. On the other hand, we have not detected a tendency of TCEP to polymerize in diluted millimolar) solutions. It is therefore likely that there the flexibility of the TCEP molecule, partially retained even in the crystal of TCEP·HCl, is responsible for kinetic stability of monomers in water solution, while the polymer represents the actual thermodynamic equilibrium state.

**Applications.** Speciation plots and log $\beta$ values presented in Table 3 indicate that TCEP complexes are rather weak, and those of TCEPO still weaker, by 1 to well over 2 orders of magnitude, as compared using log $K^C$ values. The stabilities of these complexes can be compared with those formed by DTT.[4] Direct comparisons of constants are not possible, due to the formation of multinuclear complexes by DTT and resulting differences in constant definitions. More elaborate approaches are required, based on numerical simulations of competition between TCEP and DTT for particular metal ions. We performed two kinds of these calculations. In one the DTT and TCEP were fixed at $10^{-3}$ M and metal ion concentrations were fixed at $10^{-4}$ M, and logarithms of overall concentrations of DTT- and TCEP-bound metals were plotted as a function of pH (Figure 9). In another kind of comparison, DTT and metal ion concentrations were fixed at $10^{-5}$ M, while the TCEP concentration was varied to obtain a fifty-fifty metal ion partition between the two ligands. The equal concentrations of DTT and the metal ion can be used, because 1:1 complexes dominate for DTT complexes in diluted solutions.[4] The reverse ratio of ligand concentrations is then the measure of their relative binding capabilities at a given pH. The values obtained for pH 7.4 are 268 for Ni(II), 12200 for Zn(II), 325000 for Cd(II), and 400000 for Pb(II). One can clearly conclude from these values that TCEP, being a much weaker chelator, is a safer alternative to DTT also with respect to metal-binding studies, except for Cu(II), which was redox-reactive toward either agent.

A potential disadvantage of TCEP usage, apart from the formation of binary complexes, is a distinct possibility of formation of mixed complexes with thiol-bound metal ions. Our preliminary observations indicate their presence in the TCEP−Ni(II)−glutathione system. Formation of such complexes should be tested for particular applications of TCEP. The TCEPO complexes, being much weaker those of TCEP, pose a practically negligible threat to metal−thiol studies, but quantitation of TCEPO formation, to estimate its effects on metal distribution, may be required in specific cases. This can be achieved, e.g., by using $^{31}P$ NMR in conjunction with the data given in Table 1. For the majority of cases, however, the appropriate corrections can be made by using the data presented in Table 3. The protonation value of the phosphine, 7.681, suggests the use of TCEP as a buffering component for solutions at physiological pH. It is also noteworthy that TCEP can be applied successfully in the studies of protein binding of other metal ions, e.g., Hg(II).[26,27]

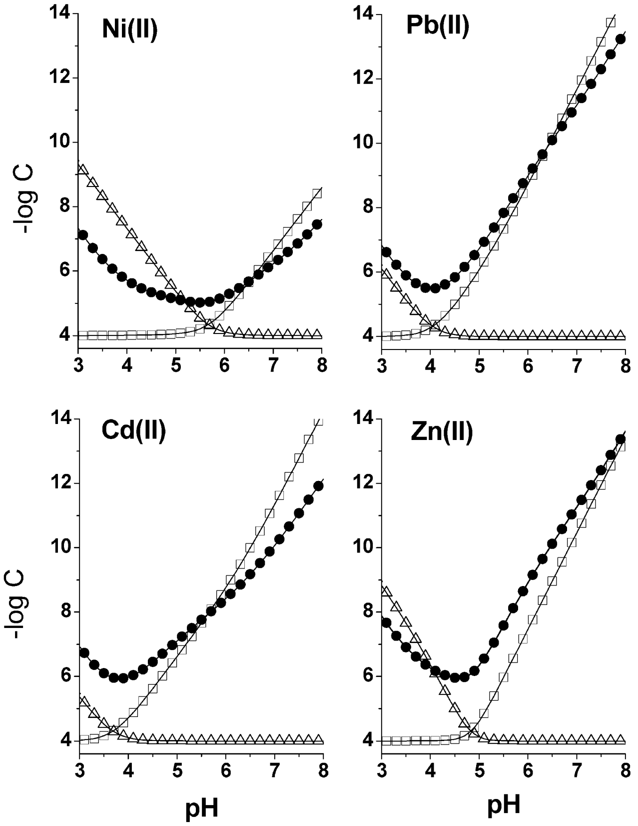

**Figure 9.** The comparison of pH-dependent metal-binding abilities of TCEP and DTT, using logarithmic competition plots, calculated for 1 mmol $dm^{-3}$ TCEP, 1 mmol $dm^{-3}$ DTT, and 0.1 mmol $dm^{-3}$ metal ions, using DTT stability constants published previously:[4] (□) $M^{2+}$; (△) M-DTT; (●) M-TCEP.

## Conclusions

The studies presented in this paper indicate that TCEP, while having interesting coordination chemistry, forms relatively weak complexes of simple stoichiometries. As a result of this it should be a much safer protecting agent for thiol compounds in the presence of metal ions than DTT. The spectroscopic and stability data provided above can serve as a basis for the planning of such experiments.

**Acknowledgment.** This work was supported by the Polish State Committee for Scientific Research (KBN), Grant

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7 T09A 079 20. The authors express their gratitude to Dr. Aleksandra Wysłouch-Cieszyńska for attracting their attention to TCEP as alternative to DTT and many helpful discussions and to Dr. Jacek Wójcik for helpful discussions of $^{31}$P NMR results.

**Supporting Information Available:** Crystallographic information files (CIF) for TCEP·HCl, $[NH_4][Zn(TCEP)]\cdot 4H_2O$, and $[NH_4][Cd(TCEP)]\cdot 6.5H_2O$. This material is available free of charge via the Internet at http://pubs.acs.org.

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