

Interactions of Aluminum(III) with Phosphates

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In order to obtain information about aluminum(III)–phosphate interactions, potentiometric measurements were carried out to characterize the complex forming properties of Al(III) with organic phosphates, phosphonates, and nucleoside-5'-monophosphates. The aluminum(III)–orthophosphate system is difficult to study due to AlPO_4 precipitation. To overcome this problem, the stability constant logarithms of the 1:1 Al(III) complexes of ligands with the same donor groups ($\log K_{1:1}$) were plotted against the basicities of the ligands ($\log K_{\text{PO}_3\text{H}}$). The resulting linear free energy relation (LFER) indicates that organic phosphates, phosphonates, and uridine-, thymidine-, and guanosine 5'-monophosphates similarly bind Al(III). Adenosine and cytidine 5'-monophosphate fall above the LFER owing to the presence of a second microform with the nucleic base protonated and a hydroxide bound to the Al(III). From the LFER the log stability constant for Al(III) binding to HPO_4^{2-} is estimated as 6.13 ± 0.05 . From the weakness of any soluble orthophosphate complexes of Al(III) we confirm the importance of citrate as the main small molecule Al^{3+} binder in the blood serum. The study includes investigation of Al(III) binding to di- and triphosphates, which bind metal ion differently than monophosphates. Structures of the complexes were supported by ^{31}P NMR measurements.

Introduction

Several ligands containing phosphate bind Al^{3+} in biological systems (but not DNA).¹ Within cells nucleoside di- and triphosphates are significant Al^{3+} binders, and their complexes have been well-characterized both by potentiometric means² and by multinuclear magnetic resonance spectroscopy.^{3–5} The stability of Al(III) with 2,3-diphosphoglycerate, a component of the red blood cell, has also been reported.⁶ In contrast to these well-characterized soluble complexes, the stabilities of Al(III) with the more weakly binding monophosphates such as adenosine 5'-monophosphate (AMP) and orthophosphate remain less certain. Much ingested Al(III) is eliminated as the insoluble phosphate complex AlPO_4 ^{7,8} or as an insoluble mixed aluminum–hydroxy–phosphate.⁹ Because of the low solubility of AlPO_4 , any soluble complexes of Al(III) and phosphate are very difficult to study,^{10–12} and their stabilities have been matters for conjecture.

One way to estimate the stability of soluble Al(III) complexes of monophosphate is to employ a linear free energy relation

(LFER) of log stability constants *versus* ligand basicity as represented by $\text{p}K_{\text{a}}$.¹³ An attempt to estimate the stability constant K_{AlPO_4} for $\text{Al}^{3+} + \text{PO}_4^{3-} \rightleftharpoons \text{AlPO}_4$ from a LFER plot of stabilities of Al(III) complexes with bidentate oxygen donor ligands assumed for PO_4^{3-} a $\text{p}K_{\text{a}} = 18.3$, the sum of the second and third dissociation constants of phosphoric acid, to obtain $\log K_{\text{AlPO}_4} = 14.1$ (after a minor correction).¹⁴ A consequence of this high stability is that the predominant phosphate complex in the blood serum is predicted to be HOAlOPO_3^- , and this complex represents the main small molecule Al^{3+} binder in the serum.

However, we feel that the $\text{p}K_{\text{a}}$ sum greatly overstates the basicity of PO_4^{3-} for Al^{3+} binding as the two summed $\text{p}K$ values are not independent, referring to proton binding at oxygen atoms that are separated by only two bonds. Hence, the derived $\log K_{\text{AlPO}_4}$ value is too high by orders of magnitude. Moreover, the straight line of the LFER contained only two phosphate ligands, adenosine 5'-diphosphate (ADP) and adenosine 5'-triphosphate (ATP), which bind Al^{3+} in six-membered chelate rings, necessarily different than the binding mode in AMP and orthophosphate.

In this research we establish the LFER for aluminum(III)–phosphate interaction with a series of ligands that have been successfully used in delineating LFER's with other metal ions.^{15,16} Several nucleoside monophosphates, such as adenosine 5'-monophosphate, cytidine 5'-monophosphate (CMP), guanosine 5'-monophosphate (GMP), thymidine 5'-monophosphate (TMP), and uridine 5'-monophosphate (UMP) have been studied. As the $\text{p}K_{\text{PO}_3\text{H}}$ values of these ligands cover a relatively

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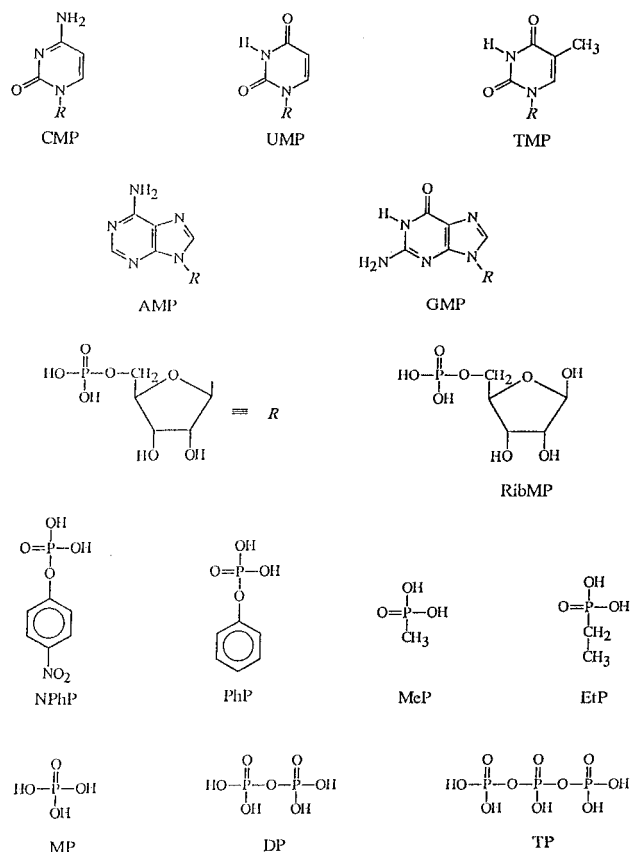


Figure 1. Structures of the ligands studied.

narrow acidity range ($pK = 6.0\text{--}6.3$), the scale has been extended to lower acidities (to $pK \sim 7.7$) by methylphosphonate (MeP) and ethylphosphonate (EtP) and to higher acidities (to $pK \sim 5$) by phenyl phosphate (PhP) and 4-nitrophenyl phosphate (NPhP). To evaluate the possible participation of the base moieties of nucleotides in metal ion binding, results have also been obtained with ribose 5'-monophosphate (RibMP). Acidity and stability constants have been determined by detailed pH-potentiometric methods. A comparative study has also been carried out with chelating diphosphate (DP) and triphosphate (TP) ligands. Structures of the ligands appear in Figure 1. Speciation results of the detailed pH-metric measurements have been confirmed by ^{31}P NMR.

Experimental Section

Reagents. Organic phosphates (CMP, GMP, TMP, UMP, RibMP, PhP, and NPhP) and inorganic phosphates (MP, DP, and TP) were the best quality sodium salts available from Sigma or Reanal Co., respectively. The organic phosphonates (MeP and EtP) were Aldrich products. Their purities were checked, and when possible, the exact concentrations of their solutions (prepared freshly each day) were determined by the Gran method.¹⁷ The Al^{3+} stock solution was prepared from recrystallized $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, and its metal concentration was determined gravimetrically via its oxinate. To prevent hydrolysis of the metal ion, the stock solution contained 0.1 mol dm^{-3} HCl. The ionic strength of all solutions studied was adjusted to 0.2 mol dm^{-3} KCl. In all cases the temperature was 25.0 ± 0.1 °C.

pH-Metric Measurements. The stability constants of the proton and Al(III) complexes of the ligands were determined by pH-metric titrations of 25.0 cm^3 samples. The concentration of the ligand was 0.004 or $0.002 \text{ mol dm}^{-3}$, and the metal ion to ligand ratios were 0:1, 1:1, 1:2, 1:4, 1:6, or 1:10. At these concentrations there is insignificant stacking between the ligand molecules.¹⁸ The titrations were performed until precipitation occurred with KOH solution of known concentration

Table 1. Proton ($\log K$) and Aluminum(III) ($\log \beta$) Stability Constants for the Complexes of Phosphates and Phosphonates at 25 °C and $I = 0.2 \text{ mol dm}^{-3}$ (KCl)

	NPhP	PhP	RibMP	MeP	EtP
$\log \beta_{\text{HA}}$	5.01(1)	5.76(1)	6.10(1)	7.43(1)	7.65(1)
$\log \beta_{\text{H}_2\text{A}}$			7.1(2)	9.56(1)	9.88(1)
$\log K_{\text{PO}_3\text{H}_2}$	<1	<1	~1	2.13	2.23
$\log K_{\text{PO}_3\text{H}^-}$	5.01	5.76	6.10	7.43	7.65
$\log \beta_{\text{AlA}}$	4.80(2)	5.29(3)	5.63(3)	6.48(3)	6.63(3)
$\log \beta_{\text{AlAH}_-1}$	1.19(2)	1.51(2)	1.69(2)	2.33(3)	2.34(2)
$\log \beta_{\text{AlAH}_-2}$	-5.5(4)	-4.5(1)	-4.83(5)	-3.91(6)	-3.93(6)
$\log \beta_{\text{AlA}_2}$				12.3(1)	12.1(2)
$\log \beta_{\text{AlA}_2\text{H}_-1}$				5.8(2)	6.1(2)
fitting ($\Delta \text{cm}^3 \cdot 10^2$) ^a	1.29	1.47	1.21	1.24	1.45
no. of points	203	227	219	216	290
$\log K_{1:1}$ ^b	4.80	5.29	5.63	6.48	6.63
$\log K_{\text{H}_-1}$	-3.61	-3.78	-3.94	-4.15	4.29
$\log K_{\text{H}_-2}$	-6.7	-6.0	-6.5	-6.2	-6.3

^a Average difference between experimental and calculated titration curves expressed in cm^3 of titrant. ^b $\text{Al}^{3+} + \text{R-O-PO}_3^{2-} \rightleftharpoons [\text{Al}(\text{R-O-PO}_3)]^+$ or $\text{Al}^{3+} + \text{R-PO}_3^{2-} \rightleftharpoons [\text{Al}(\text{R-PO}_3)]^+$.

(ca. 0.2 mol dm^{-3}) under purified argon atmosphere. Depending on the type of the ligand and the metal ion to ligand ratio, precipitation occurred in the range of $3 < \text{pH} < 7$, with the exception of the aluminum(III)-phosphonate and aluminum(III)-di- and -triphosphate systems, which could be titrated up to pH 11. Duplicate titrations were made, and the reproducibility of the titration curves was within 0.010 pH unit throughout the whole pH range. Because of the rather sluggish ligand exchange kinetics of Al(III) and the precipitation reactions, a strict criterion was applied concerning the selection of data for computer evaluation. Namely, titration points, when equilibration could not be reached in 10 min, were omitted from calculation. In this way the involvement of non-equilibrium complexation processes in calculation of the stability constants could be safely avoided.

The pH was measured with a Radiometer PHM 84 instrument with a GK2322C combined glass electrode, which was calibrated for hydrogen ion concentration according to Irving et al.¹⁹ The concentration stability constants $\beta_{\text{pqr}} = [\text{M}_p\text{A}_q\text{H}_r]/[\text{M}]^p[\text{A}]^q[\text{H}]^r$ were calculated with the aid of the computer program PSEQUAD.²⁰ For the hydroxo complexes of Al(III), the stability constants ($\log \beta$) assumed²¹ were -5.52 for $[\text{AlH}_-1]^{2+}$, -13.57 for $[\text{Al}_3\text{H}_-4]^{5-}$, -109.1 for $[\text{Al}_{13}\text{H}_-32]^{7-}$, and -23.46 for $[\text{AlH}_-4]^-$.

^{31}P NMR spectra were recorded at 25 °C on a Varian FT80A spectrometer operating at 32.203 MHz. Chemical shifts are referenced to the signal of 85% orthophosphoric acid used as an external standard. Solutions of 0.01 mol dm^{-3} Al(III) and 1:1 or 1:2 metal ion to ligand ratios were prepared in D_2O to provide an NMR lock signal. Typically 500–2000 scans were accumulated per spectrum.

Results and Discussion

1. Linear Free Energy Relation. Stoichiometries and stability constants of Al(III) complexes of several phosphates and phosphonates are listed in Table 1 and several nucleoside monophosphates in Table 2. Stability constants for the two phosphonates (see data in Table 1) conform to the linear free energy relation (LFER) as also found for other metal ions.¹⁶

Figure 2 illustrates the species distribution for the Al(III)-EtP system, typical of all the ligands listed in Table 1. The complex $[\text{AlA}]^+$ and its deprotonated forms predominate throughout the entire pH range. The bonding mode of phosphate in mononuclear complexes is not definite. The phosphate group serves mainly as a unidentate ligand with only a weak tendency

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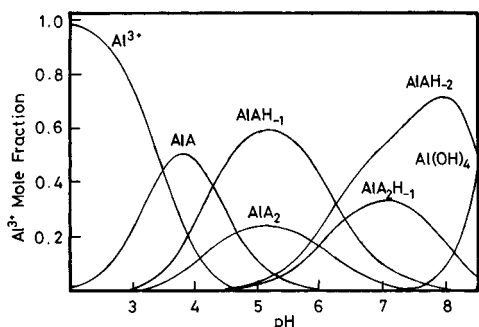
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Table 2. Proton ($\log K$) and Aluminum(III) ($\log \beta$) Stability Constants for the Complexes of Nucleoside 5'-Phosphates at 25 °C and $I = 0.2 \text{ mol dm}^{-3}$ (KCl)

	GMP	UMP	TMP	AMP	CMP
$\log \beta_{\text{HA}}$	9.42(1)	9.34(1)	9.75(1)	6.08(1)	6.11(1)
$\log \beta_{\text{H}_2\text{A}}$	15.54(1)	15.39(1)	15.99(5)	9.84(1)	10.41(1)
$\log \beta_{\text{H}_3\text{A}}$	17.96(1)		17.1(2)		
$\log K_{\text{NH}}^{\text{a}}$	2.42			3.76	4.30
$\log K_{\text{PO}_3\text{H}^-}$	6.12	6.05	6.24	6.08	6.11
$\log K_{\text{NH}}^{\text{b}}$	9.42	9.34	9.75		
$\log \beta_{\text{AlAH}}$	14.91(2)	14.83(3)	15.41(2)		9.0(2)
$\log \beta_{\text{AlA}}$	11.66(3)	11.18(2)	11.92(1)	6.14(2)	6.08(2)
$\log \beta_{\text{AlAH}_{-1}}$			5.6(2)	1.90(3)	0.39(6)
$\log \beta_{\text{AlA}_2}$		17.49(9)	18.66(6)		9.7(2)
fitting ($\Delta \text{ cm}^3 \cdot 10^2$) ^c	1.41	1.61	1.00	1.50	1.00
no. of points	124	173	163	194	159
$\log K_{1:1}^{\text{d}}$	5.49	5.49	5.66	(5.51)	(5.34)
$\log K_{\text{H}_{-1}}$	-3.25	-3.65	-3.49		
$\log K_{\text{H}_{-2}}$			-6.3		

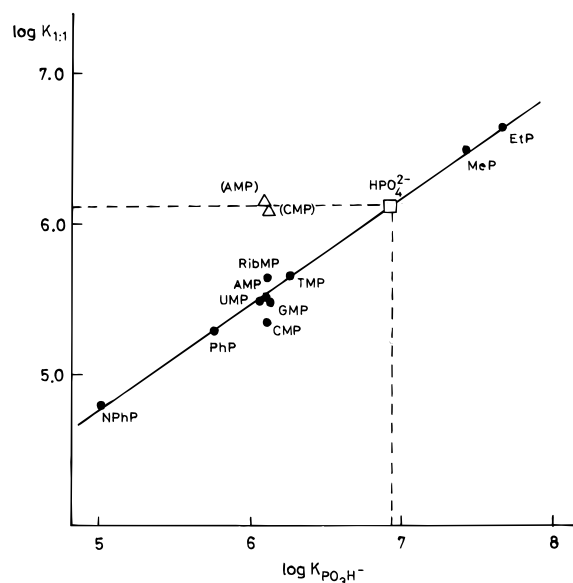
^a N7 for GMP, N1 for AMP, and N3 for CMP. ^b N1 for GMP and N3 for UMP and TMP. ^c Average difference between experimental and calculated titration curves expressed in cm^3 of titrant. ^d $\text{Al}^{3+} + \text{R-O-PO}_3^{2-} \rightleftharpoons [\text{Al}(\text{R-O-PO}_3)]^+$.

**Figure 2.** Speciation curves of the complexes formed in the Al(III)–EtP system as a function of pH: $c_{\text{Al}} = 0.001 \text{ mol dm}^{-3}$; $c_{\text{ligand}} = 0.004 \text{ mol dm}^{-3}$.

to form a four-membered chelate ring, with perhaps a greater tendency to form a six-membered ring by hydrogen bonding to phosphate oxygen from a metal ion bound hydroxide or water.¹⁵

The species $[\text{AlA}]^+$ loses a proton with $\log K_{\text{H}_{-1}} = -3.6$ to -4.3 (see Table 1). This process can be ascribed to ionization of an Al(III)-coordinated water molecule, resulting in formation of $[\text{AlAH}_{-1}]$ (or more precisely $[\text{AlA}(\text{OH})]$). A second similar process leads to formation of species $[\text{AlAH}_{-2}]^-$ ($[\text{AlA}(\text{OH})_2]^-$). In the case of the two organic phosphates precipitation occurs at $\text{pH} > 6-6.5$, even at high ligand excess (up to 1:10 metal ion to ligand ratio); it does not occur with the phosphonates until $\text{pH} \sim 9$. Hence, with phosphonates, ligand excess favors formation of the bis complex $[\text{AlA}_2]^-$ and also its mixed hydroxo species $[\text{AlA}_2\text{H}_{-1}]^{2-}$ ($[\text{AlA}_2(\text{OH})]^{2-}$). With increasing concentration, OH^- ions start to displace coordinated organic phosphonates, leading to formation of tetrahedral $[\text{Al}(\text{OH})_4]^-$. The hydrolytic tendency of complex $[\text{AlA}]^+$ decreased with an increase in basicity of the coordinating group, with a slope of 0.35 in the LFER of $\log K_{\text{H}_{-1}}$ vs $\log K_{1:1}$. This trend agrees with that obtained for a wide range of Al(III) complexes.¹⁴

Three nucleoside 5'-monophosphates, GMP, TMP, and UMP, contain a basic N donor at the nucleic base moiety: N3 for UMP and TMP and N1 for GMP; these groups are in protonated form in the formation pH range of the 1:1 Al(III) complex. The other base-N of GMP, the N7 donor, is much less basic ($\text{p}K_{\text{NH}} = 2.42$); thus, it is in deprotonated form in the pH range of Al(III) complexation. Hence, with these ligands the monophosphato complex should have a stoichiometry of $[\text{AlAH}]^{2+}$. The $\log K_{1:1}$ value characteristic for the aluminum(III)–

**Figure 3.** LFER for various organophosphates.

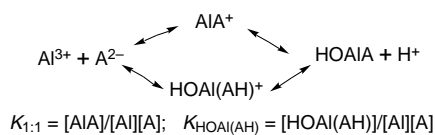
phosphate interaction and related to the reaction $\text{Al}^{3+} + \text{HA}^- \rightleftharpoons \text{AlAH}^{2+}$ can be derived as follows: $\log K_{1:1} = \log \beta_{\text{AlAH}} - \log K_{\text{HA}}$ (see Table 2). Depending on the ligand the complex $[\text{AlAH}]^{2+}$ undergoes deprotonation with $\log K_{\text{H}_{-1}} = -3.2$ to -3.6 . As for all of these ligands, $\log K_{\text{HA}} > 9.3$; this proton loss cannot be ascribed to the deprotonation of the base-NH donors but to the ionization of a coordinated water molecule, and this is in good agreement with the $\log K_{\text{H}_{-1}}$ values of the $[\text{AlA}]^+$ complexes of the other ligands (see Table 1). Thus, the complex $[\text{AlA}]^+$ of GMP, UMP, and TMP rather has a stoichiometry of $[\text{HOAl}(\text{AH})]^+$. This complex is a mixed hydroxo species, in which the base-nitrogen appears in the neutral, protonated form. As the pH is raised $[\text{AlAH}_{-1}]$ is formed with further proton loss, while in the case of some excess of ligand the bis complex $[\text{AlA}_2]^-$ can also be detected. The latter complex, however, with high uncertainty as to its formation pH range, overlaps with the beginning of precipitation. In the case of GMP, precipitation starts at $\text{pH} \sim 3.5$, even at high ligand excess. Similarly to monophosphate⁹ the precipitate is likely to be a mixed hydroxide–phosphate. The “earlier” precipitation with GMP, as compared with UMP and TMP, is most likely due to the higher hydrophobicity of the more bulky purine ring. The other purine nucleoside monophosphate AMP shows similar behavior.²

Owing to the overlapping processes of deprotonation of the base-N donor and the ionization of a coordinated water molecule of the 1:1 complex, protonated complex $[\text{AlAH}]^{2+}$ does not occur in the Al(III)–AMP system. With CMP, as the base deprotonation is somewhat shifted to a higher pH range, the protonated complex can be detected, although it is formed in very small concentration and thus its stability is loaded by high uncertainty (see Table 2). As discussed below, $[\text{AlA}]^+$ occurs in two microforms in both systems. The complex $[\text{AlAH}_{-1}]$ is a mixed hydroxo species in which the base-N donor is deprotonated: $[\text{AlA}(\text{OH})]$. The assumption of a stoichiometry of $[\text{Al}(\text{AH})(\text{OH})_2]^-$ for this species is not reasonable as the ionization of a second water molecule for the 1:1 complex occurs only at $\text{pH} > 6$, well above the dissociation pH range of the base-nitrogen. $[\text{AlA}_2]^-$ is formed in small concentration, and its “detectability” is strongly affected by the parallel precipitation reaction.

Figure 3 shows a LFER plot of $\log K_{1:1}$ vs $\log K_{\text{PO}_3\text{H}^-}$ for the five ligands in Table 1 and UMP, TMP, and GMP from Table 2. The eight ligands define an excellent straight line with slope

0.70 ± 0.02, intercept 1.29 ± 0.13, and a correlation coefficient 0.997. The slope of the line is about three times greater than that of 0.21 found for dipositive Mg²⁺ with a similar set of phosphate ligands.¹⁶ Several conclusions may be drawn from the good fit of the eight points to the straight line. The two phosphonates at the highest pK values extend the line, and their complexes are of a structure similar to the others. The hydroxy groups of the ribose in RibMP do not participate significantly in binding Al³⁺. The nucleic base nitrogens of UMP, TMP, and GMP are not involved significantly in binding Al³⁺. Since N7 of GMP participates more strongly in macrochelate formation than N7 of AMP and N3 of CMP,¹⁸ the fact that the experimental points for the last two ligands sit above the straight line of Figure 3 requires an explanation other than macrochelate formation.

The β_{AIA} stability constants given in Table 2 for AMP and CMP represent the sum over two microforms: [AIA]⁺ metal ion coordinated through the phosphate and the nucleic base deprotonated (similarly to that for the other ligands), and [HOAl(AH)]⁺ of the same net charge with a hydroxide on the metal ion and the nucleic base protonated. The two processes, nucleic base deprotonation and metal ion hydrolysis, occur in the same pH region:



The presence of the additional [HOAlAH]⁺ microform results in the points for AMP and CMP in Figure 3 sitting above the straight line. We may resolve the contributions of each microform by the following argument.

The ratio $R = [\text{HOAlAH}^+]/[\text{AIA}^+]$ may be estimated from the ratio of acidity constants to give the common deprotonated species [HOAlA]. Since the phosphate bound metal ion is remote from the proton on the nucleic base nitrogen, its acidity constant is taken as that of the free ligand (K_{NH}). For water deprotonation in the metal ion coordination sphere, log $K_{\text{H-1}}$ values in the second to last row of Table 2 for GMP and UMP are used as representative for AMP and CMP, respectively. The assumption is based on structural similarities of the corresponding ligands: GMP and AMP as well as UMP and CMP (see Figure 1). This combination of terms yields $R = 10^{-3.25}/10^{-3.76} = 10^{0.51} = 3.2$ for AMP and $R = 10^{-3.65}/10^{-4.30} = 10^{0.65} = 4.5$ for CMP. Thus, the additional [HOAlAH]⁺ microform is the dominant one. We then calculate log $K_{1:1} = \log K_{\text{AIA}} - \log(1 + R)$ and find log $K_{1:1} = 6.14 - 0.63 = 5.51$ for AMP and log $K_{1:1} = 6.08 - 0.74 = 5.34$ for CMP. These estimated values for log $K_{1:1}$ fall close to the LFER line in Figure 3 and, because they are sensitive to inputs, appear in parentheses near the bottom of Table 2.

2. Orthophosphate Binding of Al³⁺. The LFER of Figure 3 with the slope and intercept given above may be used to estimate the stability constant for the reaction $\text{Al}^{3+} + \text{HPO}_4^{2-} \rightleftharpoons \text{AlOPO}_3\text{H}^+$, analogous to the other reactions in the figure. Since log $K_{\text{H}_2\text{PO}_4} = 6.63$ refers to the loss of one of two protons from H_2PO_4^- , the intrinsic pK_a = 6.63 + log 2 = 6.93. This value yields log $K_{1:1} = 6.13 \pm 0.05$ for the complexation reaction; this is the quantitative measure of the Al(III) binding ability of hydrogenphosphate as compared with that of the organic phosphates. We also tried to describe the Al(III)–MP system by direct pH–potentiometric measurements. The best fit between the experimental and the calculated titration data measured in the pH range 2–3.5 was obtained with a log K_{AlAH}

Table 3. Proton (log K) and Aluminum(III) (log β) Stability Constants for the Complexes of Orthophosphate at 25 °C and $I = 0.2 \text{ mol dm}^{-3}$ (KCl)

	ref 11 ^a	ref 10 ^b	ref 12 ^a	this work ^c
log β _{HA}	11.39(1)	11.80(1)	11.54(1)	11.48(1)
log β _{H₂A}	18.00(1)	18.31(1)	18.22(1)	18.11(1)
log β _{H₃A}	19.96(1)	19.51(1)	20.22(1)	19.97(1)
log K _{H₃A}	1.96	1.20	2.00	1.86
log K _{H₂A}	6.61	6.51	6.68	6.63
log K _{HA}	11.39	11.80	11.54	11.48
log β _{AlAH₂}	22.25(4)	26.18(1)	20.93(4)	
log β _{AlAH}	19.1(1)	23.25(1)	17.79(1)	17.60(f) ^d
log β _{AlA}	15.7(4)		15.32(5)	
log β _{AlA₂H}		37.95(1)		
log β _{Al₂A}	20.9(1)		18.72(5)	16.65(18)
log β _{Al₂AH₋₁}				14.21(4)
log β _{Al₂AH₋₂}	15.80(6)		12.58(5)	
log β _{Al₂AH₋₃}	6.7(2)			7.42(4)

^a $I = 0.15 \text{ mol dm}^{-3}$ (NaCl); $t = 37 \text{ °C}$. ^b $I = 0.15 \text{ mol dm}^{-3}$ (NaCl); $t = 25 \text{ °C}$. ^c $I = 0.2 \text{ mol dm}^{-3}$ (KCl); $t = 25.0 \text{ °C}$. ^d Fixed value from LFER plot.

= log β_{AlAH} – log $K_{\text{HA}} = 5.43$, which is about 0.7 log unit lower than the one determined by the LFER method. As direct potentiometric measurements are limited in pH range and not accurate at higher pH, when precipitation and slow oligomerization reactions occur, we think the constant obtained from the LFER approach is the more reliable. Hence, the value of this constant was fixed, while the constants of the other species were refined in the calculation. The results of the calculation are listed in Table 3 together with speciation results reported earlier.^{10–12}

It was found that the species [AlAH]⁺ and [Al₂A]³⁺ can substitute for each other to a great extent in the calculation procedure, owing to the similar pH dependence of their formation reactions: $\text{Al}^{3+} + \text{H}_2\text{A}^- \rightleftharpoons \text{AlAH}^+ + \text{H}^+$ and $\text{Al}^{3+} + \text{AlAH}^+ \rightleftharpoons \text{Al}_2\text{A}^{3+} + \text{H}^+$. It is likely that this fact also played a role in high uncertainties and contradictions in literature speciation interpretations. The fitting parameter characterizing the fit between the experimental and calculated titration data hardly increased (from 0.0047 to 0.0053 cm³), when calculation was made with the fixed value for [AlAH]⁺ obtained from LFER. This strongly supports the above assumption concerning the interchangeability of the species [AlAH] and [Al₂A]. The LFER treatment seems to be suitable for separating these two parallel processes and thus for describing the aluminum(III)–orthophosphate interaction with greater certainty. The speciation model given in Table 3 is confirmed by ³¹P NMR measurements (see Table 5). In the pH range 1.5 < pH < 2.5 two significantly different broad signals could be detected: at –8 and –13 ppm. On the basis of the relative intensity of the two peaks, the former should probably belong to complex [AlAH]⁺, while the latter to [Al₂A]³⁺.

When the hydrolysis constants (log $K_{\text{H-1}}$) of the Al(III) complexes studied are depicted as a function of their log $K_{1:1}$ stability constants (see above), a straight line is obtained and a log $K_{\text{H-1}} = -4.08 \pm 0.04$ can be calculated for the corresponding reaction $\text{AlAH}^+ \rightleftharpoons \text{HOAl(AH)} + \text{H}^+$ of orthophosphate. This species could not be detected by direct pH–metry as it was formed at the boundary of precipitation. However, if a species [AlA] (= [HOAl(AH)]) is assumed and fixed with a stability constant log β_{AlA} = log β_{AlAH} – log $K_{\text{H-1}} = 17.6 - 4.1 = 13.5$ in evaluation of the titration data the fitting parameter remains the same (and this species is formed in a maximum 10% at the highest measured pH). We can obtain a second deprotonation constant through a similar handling of the log $K_{\text{H-2}}$ data of the aluminum(III)–organophosphate complexes. The uncertainty of log $K_{\text{H-1}}$ values (see the last rows of Tables

1 and 2), however, are much larger, and thus there is no unambiguous dependence of $\log K_{1:1}$ vs $\log K_{H-2}$. To estimate the second deprotonation constant for the aluminum(III)–orthophosphate complex the average value $\log K_{H-2} = -6.3 \pm 0.2$ was taken. This treatment yields a constant $\log \beta_{\text{AlAH}_{-1}} = 13.5 - 6.3 = 7.2$ for orthophosphate. This species, for which we do not have any direct evidence, would have a binding mode of $[(\text{HO})_2\text{Al}(\text{AH})]$. It is very likely, however, that this second hydrolytic step is accompanied by the parallel deprotonation of the ligand; thus, the species can exist in another microform too: $[\text{HOAlA}]$.

We may employ the linear free energy relation as the centerpiece for estimating additional equilibrium constants. Using the same slope and intercept in the LFER plot of Figure 3 and a $\text{p}K = 11.48$ for deprotonation of HPO_4^{2-} , we find for $\text{Al}^{3+} + \text{PO}_4^{3-} \rightleftharpoons \text{AlPO}_4$, $\log K_{\text{AlPO}_4} = 9.3$. However, this is a minimum value as the LFER plot refers to ligands with a 2–charge and PO_4^{3-} is 3– charged. To allow for the charge effect, we add two log units to obtain $\log K_{\text{AlPO}_4} \sim 11.3$. This value is still 3 orders of magnitude less than an earlier estimate of 14.1¹⁴ and constitutes the major difference between that and our analysis. Now, we apply the hydrolysis constant $\log K_{H-1} = -4.08 \pm 0.04$ obtained above to estimate the stability constant for $[\text{HOAlPO}_4]$: $\log \beta_{\text{HOAlPO}_4} = 11.3 - 4.1 = 7.2$. This is the same value that was obtained through taking into account the consecutive hydrolysis constants $\log K_{H-1}$ and $\log K_{H-2}$ of model organic phosphates. We strongly believe that the two mononuclear mixed hydroxo species have much higher importance in Al(III) binding under physiological conditions, when Al(III) is only at the $\mu\text{mol dm}^{-3}$ level. In such a dilute solution formation of dinuclear species, like $[\text{Al}_2\text{A}]^{3+}$, $[\text{Al}_2\text{AH}_{-1}]^{2+}$, and $[\text{Al}_2\text{AH}_{-3}]$ (see Table 3), is strongly suppressed. We do not think that the stability constant determined for the dinuclear complex $[\text{Al}_2\text{AH}_{-3}]$ in the pH range 2–3.5 can be safely used in speciation calculation for physiological pH range.

By an entirely different argument, but in agreement with an earlier result,⁹ this research concludes that the monophosphate complex of Al(III) contributes less importantly to low molecular weight fractions in the blood plasma. These results seem to reaffirm citrate as the main low molecular weight bearer of Al^{3+} in the serum with, however, about 85% of the Al(III) binding to the protein transferrin.^{1,9,22,23} These conclusions, based upon stability constants, agree with direct observation by ¹H NMR spectroscopy of Al^{3+} binding to citrate in human blood plasma.²⁴ The complete description of Al(III) speciation in serum, however, needs further studies, among others the inclusion of ternary complex formation between citrate and phosphate, the two most important potential low molecular weight Al binders. Such investigations are now in progress in our laboratories.

3. Di- and Triphosphate Binding. Compared to monophosphate, diphosphate and triphosphate form much stronger complexes with Al(III). The first may chelate Al(III) through a low-stability four-membered ring, while the last two bind *via* adjacent phosphate oxygens to form much more stable six-membered chelates.

The stability constants obtained by the joint evaluation of titration curves of the Al(III)–DP and Al(III)–TP systems are listed in Table 4 together with their respective protonation constants with $\text{p}K > 1$. Table 4 shows that both DP and TP

Table 4. Proton ($\log K$) and Aluminum(III) ($\log \beta$) Stability Constants for the Complexes of Diphosphate and Triphosphate at 25 °C and $I = 0.2 \text{ mol dm}^{-3}$ (KCl)

	DP	TP
$\log \beta_{\text{HA}}$	8.23(1)	7.76(1)
$\log \beta_{\text{H}_2\text{A}}$	14.11(1)	13.10(1)
$\log \beta_{\text{H}_3\text{A}}$	15.78(1)	14.80(1)
$\log K_{\text{H}_3\text{A}}$	1.67	1.70
$\log K_{\text{H}_2\text{A}}$	5.87	5.34
$\log K_{\text{HA}}$	8.23	7.76
$\log \beta_{\text{AlAH}_2}$	18.69(3)	18.07(5)
$\log \beta_{\text{AlAH}}$	17.03(3)	16.65(4)
$\log \beta_{\text{AlA}}$	13.74(3)	13.15(4)
$\log \beta_{\text{AlAH}_{-1}}$	7.41(4)	6.53(5)
$\log \beta_{\text{AlA}_2\text{H}}$	25.64(3)	24.43(5)
$\log \beta_{\text{AlA}_2}$	19.77(1)	19.14(2)
fitting ($\Delta \text{ cm}^3 \cdot 10^2$) ^a	0.55	1.46
no. of points	275	299
$\log K_{\text{AlAH}_2}$	4.58	4.97
$\log K_{\text{AlAH}}$	8.80	8.89
$\log K_{\text{AlA}_2}$	6.03	5.99
$\log (K_{\text{AlA}}/K_{\text{AlA}_2})$	7.71	7.16
$\log K_{(\text{Al} + \text{H}_3\text{A} \rightleftharpoons \text{AlAH}_2 + \text{H})}$	2.91	3.27
$\log K_{(\text{Al} + \text{H}_3\text{A} \rightleftharpoons \text{AlAH} + 2 \text{H})}$	1.25	1.85
$\log K_{(\text{Al} + \text{H}_2\text{A} \rightleftharpoons \text{AlAH} + \text{H})}$	2.92	3.55
$\log K_{(\text{Al} + \text{H}_2\text{A} \rightleftharpoons \text{AlA} + 2 \text{H})}$	-0.37	0.05
$\log K_{(\text{AlA} + \text{HA} \rightleftharpoons \text{Al}_2\text{H})}$	3.67	3.52
$\log K_{(\text{AlA} + \text{HA} \rightleftharpoons \text{AlA}_2 + \text{H})}$	-2.20	-1.77

^a Average difference between experimental and calculated titration curves expressed in cm^3 of titrant.

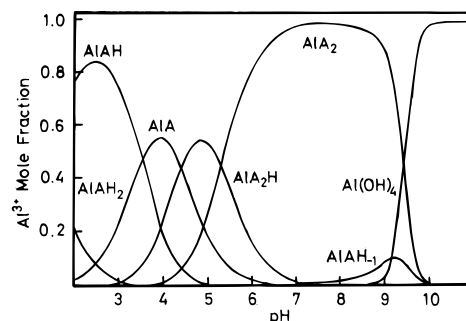


Figure 4. Speciation curves of the complexes formed in the Al(III)–TP system as a function of pH: $c_{\text{Al}} = 0.001 \text{ mol dm}^{-3}$; $c_{\text{ligand}} = 0.004 \text{ mol dm}^{-3}$.

form complexes of the same stoichiometries. The stability constants are somewhat lower for TP than for DP. However, if the basicity adjusted stability constants, which take into account the differences in basicity of the coordinating donor groups, are compared, the Al(III) complexes of TP are the more stable, as is expected on the basis of the higher charge and larger number of potential coordination sites of TP. As is reflected in the $\log(K_{\text{AlA}}/K_{\text{AlA}_2})$ values, the coordination of a second molecule is a little more hindered for TP due to the higher electrostatic repulsion between the more highly charged species of TP. The species distributions for the two systems are similar: an illustration of the Al(III)–TP system depicted in Figure 4 shows predominance of the 1:2 complex in neutral solutions.

³¹P NMR measurements support the pH-metric speciation results. The ³¹P chemical shifts obtained at different experimental conditions (pH, Al/A ratio) and their likely assignments are summarized in Table 5. At pH 2.1 free DP shows a sharp peak at -10.9 ppm (the two phosphorus atoms are magnetically equivalent). Binding of Al(III) to DP splits the signal into two broad peaks: at -10.9 and -13.2 ppm . The coordination of Al(III) removes magnetic equivalence of the P atoms in DP, resulting in overlapping signals which merge into broad bands. The resonance at -13.2 ppm presumably belongs to the chelated

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Table 5. ^{31}P NMR Parameters of Aluminum(III)–Phosphate Systems

pH	Al ³⁺ /A	$\delta(^{31}\text{P})$	assignment
Aluminum(III)–Diphosphate			
2.1	0/2	-10.8	free ligand
	1/2	-10.9 (broad)	free ligand + AlAH ₂ (monodentate)
		-13.2 (broad)	AlAH (chelate)
4.5	0/2	-10.6	free ligand
	1/2	-10.7	free ligand
		-11.7 (broad)	AlA ₂ H (chelate + monodentate)
7.5	0/2	-7.4	free ligand
	1/2	-9.2	AlA ₂ (chelate)
	2/2	-7.6 (broad) ^a	free ligand
		-8.5 (broad)	AlAH ₋₁
		-9.1 (weak)	AlA ₂ (chelate)
Aluminum(III)–Triphosphate			
2.1	0/2	-10.5, -11.1	free α,γ -P
		-22.6, -23.2, -23.8	free β -P
		-10.9, -11.4	free α,γ -P
	1/2	-22.4, -23.0, -23.6	free β -P
		-12.4 (broad)	AlAH (chelate)
		-20.5 (broad)	AlAH (chelate)
	2/2	-12.6 (broad)	AlAH (chelate)
		-20.7 (broad)	AlAH (chelate)
		-20.7 (broad)	AlAH (chelate)
4.5	0/2	-10.2, -10.8	free α,γ -P
		-22.4, -23.1, -23.7	free β -P
		-10.3, -11.1	AlA (chelate)
	1/2	-22.4, -23.1, -23.7	AlA (chelate)
		-17.5 (broad)	AlA ₂ H
		-7.0, -7.6	free α,γ -P
7.5	0/2	-20.8, -21.4, -22.1	free β -P
		-9.2, -9.5	AlA ₂ (chelate)
	1/2	-17.4, -17.9, -18.4	AlA ₂ (chelate)
Aluminum(II)–Phosphate			
1.4	2/2	-0.3	free ligand
		-8.2 (broad)	AlAH
		-14.4 (broad)	Al ₂ A
2.3	2/2	-8.1 (broad)	AlAH
		-12.6 (broad)	Al ₂ A
2.2	1/2	-1.8	free ligand
		-7.9 (broad)	AlAH
		-13.2 (broad)	Al ₂ A

^a Partial disproportionation of the 1:1 complex to species AlA₂ and Al(OH)₄.

species [AlAH]. A similar upfield shift of the DP phosphorus was reported also by Karlik et al.³ The other species [AlAH₂]⁺ present in significant concentration at pH ~ 2.1 probably gives the broad peak at -10.9 ppm with strong overlap with that of the free DP. In this double-protonated species the ligand coordinates only through one of the terminal phosphates in a unidentate way or *via* a four-membered chelate; the other phosphate terminus remains free. This may explain the little shift of the phosphorus resonance with respect to the free ligand. When the pH is increased to pH 7.5, the free ligand is mostly in the HP₂O₇³⁻ form, and the ^{31}P NMR spectrum shows a sharp singlet at -7.4 ppm. When Al(III) binds to DP at this pH at 1:2 ligand to metal ion ratio, the ^{31}P signal is broadened

and shifted upfield to -9.2 ppm. This peak could belong to the chelated bis complex [AlA₂]⁵⁻. In equimolar solution, where the speciation diagram shows the presence of a single mixed hydroxo species [AlAH₋₁]²⁻ ($\delta = -8.5$ ppm), the occurrence of the resonance at -9.1 ppm (characteristic of species [AlA₂]⁵⁻) suggests that a partial disproportionation of the 1:1 complex takes place to complexes [AlA₂]⁵⁻ and [Al(OH)₄]⁻. It is confirmed by the detection of the broad peak of free DP at -7.6 ppm. In the intermediate pH (~4.5) the broad signal at -11.7 ppm, ~1 ppm upfield to the free DP, can be ascribed to species [AlA₂H]⁴⁻. In this complex one ligand probably coordinates in a chelating mode, while the other only in a unidentate (or in a four-membered chelating) way. This may explain the broadening of the free ligand signal, due to an overlap between resonances of this weakly coordinated phosphate and the free phosphate (see above). In summary, we suggest that deprotonation of H₂P₂O₇²⁻ to HP₂O₇³⁻ shifts the ^{31}P resonance downfield, while complexation with Al(III) results in 1–2.5 ppm upfield shifts compared to the peaks of the free ligand; this upfield shift is somewhat larger for the 1:2 complexes than for the 1:1 complexes.

The effect of Al(III) on TP is still more complex: the ligand itself gives two peaks. The signal of the central β -P is a triplet at -23.2 ppm ($^2I_{\text{PP}} = 19.4$ Hz) due to coupling with the two neighboring terminal phosphorus atoms, while the terminal α -Ps give a doublet at -10.8 ppm. Binding of Al(III) to TP at pH 2.1 broadens the ^{31}P signals and shifts them to -12.5 and -20.5 ppm, both at 2:2 and 1:2 metal ion to ligand ratios. This may correspond to the formation of a single species AlAH⁻ under these conditions; in the case of ligand excess the peaks of the free ligand are also well-observed. At pH 7.5, when the ligand is partly in the fully deprotonated form, deprotonation shifts the resonances of the terminal α -Ps 3.5 ppm downfield to -7.3 ppm, while those of the central β -P shift 1.8 ppm upfield to 21.4 ppm with respect to acidic pH. In the presence of Al(III) these peaks are shifted further; the signal of the central P atom appears as a broad triplet centered at 17.9 ppm, while that of the terminal P atoms as a doublet at -9.3 ppm. These data are in accordance with the pH-metric result: at pH 7.5 TP is found mostly in the complex [AlA₂]⁷⁻. In the intermediate pH (=4.5) the ^{31}P NMR spectrum is even more complex (see Table 5) as ^{31}P signals of both the 1:1 and 1:2 complexes can be observed. It appears that coordination of Al(III) to DP and TP broadens the ^{31}P NMR signals and shifts the resonances of the terminal phosphorus atoms upfield, while those of the central phosphorus of TP move downfield.

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