Interaction of Pyrophosphate Ion with Di-, Tri- and Tetra-amines in Aqueous Solution: A Potentiometric and Calorimetric Study

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Successive macroscopic pK_a values have been determined potentiometrically for the conjugate acids of spermidine (4-azaoctane-1,8-diamine), spermine (4,9-diazadodecane-1,12-diamine) and some acylic diamines at various ionic strengths in the absence and presence of tetrasodium pyrophosphate. The stability of the amine/pyrophosphate adducts have been estimated on the basis of effects of the pyrophosphate ion on the apparent acidity constants of the amines. Stoichiometry and thermodynamics of adduct formation have been elucidated from microcalorimetric measurements.

Inorganic pyrophosphatase (EC 3.6.1.1) is an enzyme that catalyzes the hydrolysis of pyrophosphate ion (PP_i^{4-}) to orthophosphate ions. The structural and kinetic properties of various microbial pyrophosphatases have been well established.[1-8] The catalytic activity of inorganic pyrophosphatase of *Streptococcus faecalis*, for example, exhibits an absolute requirement for Mg^{2+} ion,[9] which is necessary for the formation of substrate complexes, $[Mg(PP_i)]^{2-}$ and $[Mg_2(PP_i)]$. Moreover, Mg^{2+} ion acts as an allosteric activator.[7,10] Recently, significant catalysis was observed by the acyclic tri- and tetra-amines, spermidine (4-azaoctane-1,8-diamine, **1**) and spermine (4,9-diazadodecane-1,12-diamine, **2**) even in the absence of divalent metal ions.[11] This suggests that protonated polyamines are able to complex with the pyrophosphate ion. Application of resin competition methodology[12] at pH 7.5 gave values of 6.4×10^2 and 2.7×10^3 dm^3 mol^{-1} for the apparent stability constants of the 1:1 adducts of **1** and **2** with PP_i.[11] This paper is aimed at elucidating the nature and strength of these interactions.

Results and discussion

Table 1 records the successive macroscopic pK_a values of the conjugate acids of spermidine (**1**), spermine (**2**), propane-1,3-diamine (**3**), butane-1,4-diamine (**4**), and pentane-1,5-diamine (**5**). The pK_a values were determined potentiometrically at various ionic strengths adjusted with tetramethylammonium chloride. All the pK_a values increase with increasing ionic strength, the increment being more marked with polyprotonated than with monoprotonated species; the pK_a values of mono-, di-, tri- and tetracations are increased by 0.2, 0.4, 0.7 and 0.8 units, respectively, when the ionic strength was increased from 0.1 to 1 mol dm^{-3}. The effect of added sodium nitrate was observed to be slightly larger. Apparently, an ionic environment stabilizes the fully protonated molecule relative to less charged species. As seen in Table 1, the values are compatible with those reported in the literature.

1	$H_2N(CH_2)_3NH(CH_2)_4NH_2$
2	$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$
3	$H_2N(CH_2)_3NH_2$
4	$H_2N(CH_2)_4NH_2$
5	$H_2N(CH_2)_5NH_2$

The observed pK_a values of mono- and di-hydrogen pyrophosphate ions are presented in Table 2. These values are rather insensitive to the concentration of tetramethylammonium chloride. Thus, interactions between pyrophosphate and tetramethylammonium ions appear to be negligible. In comparison, the pK_a value of monohydrogen pyrophosphate ion was observed to decrease by more than one unit on going from I = 0.1 mol dm^{-3} to I = 1 mol dm^{-3}, when the ionic strength was adjusted with sodium nitrate instead of tetramethylammonium chloride. This enhanced acidity probably results from complexation of the sodium ion with the pyrophosphate tetraanion. For the 1:1 complex a logarithmic stability constant of 0.8 has been reported.[21]

Table 3 summarizes the equilibrium data obtained with equimolar mixtures of pyrophosphate ion and organic amines. It can clearly be seen that the presence of a fully protonated amine significantly increases the apparent acidity of mono- (HPP_i^{3-}) and di-hydrogen ($H_2PP_i^{2-}$) pyrophosphate ions, the effect on the pK_a value of the former species being more prominent. Simultaneously, the apparent acidity of the protonated amines decreases, and the effect is most pronounced for the polycharged species. The most likely explanation for these changes in apparent pK_a values

Table 1. Macroscopic pK_a values of the conjugate acids of spermidine (**1**), spermine (**2**), propane-1,3-diamine (**3**), butane-1,4-diamine (**4**), and pentane-1,5-diamine (**5**) at various ionic strengths at 298.2 K.[a]

Compd.	*I*/mol dm^{-3}	$pK_a(AH_4^{4+})$	$pK_a(AH_3^{3+})$	$pK_a(AH_2^{2+})$	$pK_a(AH^+)$
1[b]	0.10		8.15	9.74	10.24
	0.50		8.77	10.24	10.88
	1.00		8.93	10.30	10.86
2[c]	0.10	7.91	8.68	10.21	10.56
	0.50	8.52	9.14	10.51	10.76
	1.00	8.69	9.31	10.65	10.80
3[d]	0.10			8.80	10.57
	0.50			9.00	10.62
	1.00			9.18	10.65
4[e]	0.10			9.45	10.77
	0.50			9.67	10.90
	1.00			9.77	10.95
5[f]	0.10			9.85	11.00
	0.50			10.05	11.07
	1.00			10.10	11.14

[a]Ionic strength adjusted with tetramethylammonium chloride. [b]Lit. 8.34, 9.81, 10.89 (potent., I = 0.1 mol dm^{-3});[13] 8.25, 9.71, 10.90 (^{13}C NMR, I = 0);[14] 8.51, 10.06, 11.16 (potent., I = 0.1 mol dm^{-3});[15] 8.56, 9.98, 10.95 (potent., I = 0.15 mol dm^{-3}).[16] [c]Lit. 7.96, 8.85, 10.02, 10.80 (potent., I = 0.1 mol dm^{-3});[13] 8.03, 9.04, 10.27, 10.57 (potent., I = 0.1 mol dm^{-3});[15] 7.97, 9.04, 10.12, 10.94 (potent., I = 0.15 mol dm^{-3}).[16] [d]Lit. 8.85, 10.62 (potent., I = 0.1 mol dm^{-3}).[17] [e]Lit. 9.61, 10.82 (potent., I = 0.1 mol dm^{-3}, T = 293.2 K).[18] [f]Lit. 10.02, 10.96 (potent., I = 0.1 mol dm^{-3}, T = 293.2 K).[18]

is adduct formation between negatively charged pyrophosphate ions and positively charged protonated amines [eqn. (1)]. The adduct may be expected to be more stable the

$$H_mPP_i^{(4-m)-} + AH_n^{n+} \rightleftharpoons AH_{n+m}PP_i^{(4-m-n)-} \quad (1)$$

higher the charge of the interacting ions. Accordingly, the ability of pyrophosphate ions to form adducts with fully protonated amines, [the predominant ionic forms at pH < $pK_a(HPP_i^{3-})$], decreases in the order: $PP_i^{4-} > HPP_i^{3-} > H_2PP_i^{2-} > H_3PP_i^{+}$. Since the product forms a more stable adduct than the reactant, each of the consecutive deprotonation steps is enhanced. For the same reason the apparent pK_a value of HPP_i^{3+} is changed more than that of $H_2PP_i^{2-}$. Analogously, the ability of protonated amines to form adducts with PP_i^{4-}, which prevails at pH > $pK_a(AH_4^{4+})$, decreases in the order: $AH_n^{n+} > AH_{n-1}^{(n-1)}$. In other words, the reactant in each dissociation step forms a more stable pyrophosphate adduct than does the product, and hence the apparent acidity is reduced.

Table 2. Macroscopic pK_a values of mono- and di-hydrogen pyrophosphate ions at various ionic strengths at 298.2 K.[a]

I/mol dm^{-3}	$pK_a(H_2PP_i^{2-})$	$pK_a(HPP_i^{3-})$
0.10[b]	6.41	8.78
0.50	6.31	8.75
1.00	6.34	8.90

[a]The ionic strength was adjusted with tetramethylammonium chloride. [b]Lit. 6.12, 8.93 (potent., I = 0.1 mol dm^{-3} with Me_4NCl),[19] 6.12, 8.95 (potent., I = 0.1 mol dm^{-3} with Me_4NBr).[20]

The data in Table 3 allow the following conclusions to be made. (i) The ΔpK_a values decrease on going from an ionic strength of 0.1 to 0.5 mol dm^{-3}, and change only slightly thereafter. This means that $H_mPP_i^{(4-m)-}/AH_n^{n+}$-adducts are destabilized by high electrolyte concentrations, which is expected on the basis of the electrostatic nature of the interaction. (ii) The $\Delta pK_a(AH_3^{3+})$ value of spermidine (**1**), and the $\Delta pK_a(AH_4^{4+})$ and $\Delta pK_a(AH_3^{3+})$ values of spermine (**2**) are all markedly positive, in accordance with the strong interaction of polycharged amines with PP_i^{4-}. (iii) The dication of propane-1,3-diamine (**3**) interacts with PP_i^{4-} more strongly than those of the other diamines studied. This interaction with PP_i^{4-} thus seems to become weaker when the number of methylene groups between the protonated nitrogen atoms is increased. It is interesting to note that the dication of spermidine (**1**) appears to interact with PP_i^{4-} more strongly than does the dication of spermine (**2**). In fact, the affinity of diprotonated spermidine for PP_i^{4-} is comparable to that of diprotonated propane-1,3-diamine. For the protonation of the polyamines **1** and **2**, two different binding modes have been suggested. From the ^{13}C NMR shift data of Kimberley and Goldstein,[14] and the 2D ^{1}H, ^{13}C NMR spectroscopic studies of Aikens *et al.*[22] one can conclude that all the nitrogen atoms of **1** are involved in dication formation. Protonation of the terminal amino groups is only moderately favoured. In contrast, the ^{13}C NMR spectroscopic results of Delfini *et al.*[23] and the ^{15}N NMR spectroscopic results of Takeda *et al.*[15] suggest a different tautomeric structure for the dications of **1** and **2**;

Table 3. pK_a values for pyrophosphoric acid and the conjugate acids of organic amines in their equimolar mixtures (0.01/0.01 mol dm^{-3}) at 298.2 K.[a]

A	*I*/mol dm^{-3}	$\Delta pK_a(H_2PP_i^{2-})$	$\Delta pK_a(HPP_i^{3-})$	$\Delta pK_a(AH_4^{4+})$	$\Delta pK_a(AH_3^{3+})$	$\Delta pK_a(AH_2^{2+})$	$\Delta pK_a(AH^+)$
1	0.10	−0.6	−1.2		+1.3	+0.7	+0.1
	0.50	−0.1	−0.8		+0.5	+0.4	0
	1.00	−0.2	−0.6		+0.5	+0.3	0
2	0.10	−1.0	−1.4	+1.5	+1.3	0	0
	0.50	−0.5	−1.0	+0.9	+0.6	+0.2	0
	1.00	−0.4	−1.0	+0.9	+0.5	0	0
3	0.50	−0.5	−0.7			+0.5	+0.1
	1.00	−0.3	−0.6			+0.5	+0.2
4	0.50	−0.4	−0.5			+0.3	+0.1
	1.00	−0.2	−0.5			+0.2	+0.1
5	0.50	−0.4	−0.3			+0.2	+0.1
	1.00	−0.3	−0.3			+0.1	+0.1

[a]The ΔpK_a values reported are the difference between the pK_a values observed in the presence and in the absence of the cosolute (PP_i/A). The ionic strength was adjusted with tetramethylammonium chloride.

only the terminal amino groups undergo protonation. The relatively high affinity of diprotonated spermidine for PP_i^{4-} may be accounted for by the former binding mode. In the case of spermine, with two imino groups in addition to the terminal amino groups, simultaneous protonation of the two adjacent nitrogen atoms will be, statistically, less favourable. (iv) The pK_a values of monoprotonated amines are not markedly influenced by the presence of PP_i^{4-}, suggesting that neutral and monocharged amines interact only weakly with PP_i^{4-}.

Calorimetric studies on the interaction of PP_i^{4-} with fully protonated amines, **1** and **2**, aid the elucidation of the stoichiometry of the adduct formation. Table 4 summarizes the enthalpies of interaction determined by mixing solutions of tetrasodium pyrophosphate and solutions of spermidine trihydrochloride and spermine tetrahydrochloride at different concentration ratios. The data show that the enthalpy at $[Na_4PP_i]/[AH_nCl_n] = r$ is nearly equal to that at $[Na_4PP_i]/[AH_nCl_n] = 1/r$, provided that the total concentration, $[Na_4PP_i] + [AH_nCl_n]$, remains constant. This suggests that the adducts contain PP_i^{4-} and AH_n^{n+} in a 1:1 ratio. Although the contributions of intermolecular proton transfer reactions from AH_n^{n+} to PP_i^{4-} cannot be strictly excluded, they are presumably of minor importance. As seen from Tables 1–3, the apparent pK_a values of AH_n^{n+} and HPP_i^{4-} differ in their mixtures by more than 1 unit.

Table 4. Enthalpies of interaction of pyrophosphate tetraanion with fully protonated spermidine (**1**) and spermine (**2**) in aqueous solution at 298.2 K.[a]

[Amine]/mol dm^{-3}	$[PP_i]$/mol dm^{-3}	ΔH/J
Spermidine		
0.0067	0.0133	0.582
0.0133	0.0067	0.550
0.0067	0.0333	0.750
0.0333	0.0067	0.779
0.0067	0.0667	0.781
0.0667	0.0067	0.863
Spermine		
0.0067	0.0133	0.753
0.0133	0.0067	0.659
0.0067	0.0333	0.988
0.0333	0.0067	0.848
0.0067	0.0667	1.054
0.0667	0.0067	0.987

[a]The concentrations reported are total concentrations after mixing. The enthalpy values refer to a final volume of 6.0 cm^3. No background electrolyte was employed.

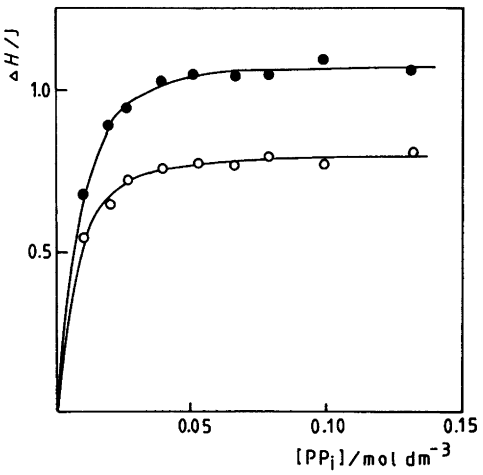

Fig. 1. Enthalpies of interaction of PP_i^{4-} with fully protonated spermidine (○) and spermine (●) in excess PP_i^{4-} at 298.2 K. The concentration of the protonated amine was 6.7×10^{-3} mol dm^{-3} and the final volume of the reaction mixture was 6.0 cm^3. No background electrolyte was employed.

Fig. 1 shows the enthalpy of interaction *vs.* the concentration of PP_i^{4-}, when the latter species was in excess. With both spermidine trihydrochloride and spermine tetrahydrochloride the enthalpy is independent of the pyrophosphate concentration when $PP_i^{4-} > 0.05$ mol dm^{-3}. This indicates that **1** and **2**, under these conditions, are entirely converted into pyrophosphate adducts. Since the ratio of these PP_i^{4-}-independent enthalpy values for **1** and **2** is almost exactly 3:4, the nitrogen atoms are most probably equally involved in the adduct formation. The standard molar enthalpies for the formation of PP_i^{4-}-adducts of fully protonated spermidine and spermine are 19.7 and 26.5 kJ mol^{-1}, respectively.

Bearing in mind the results of the calorimetric measurements, the stability constants of the AH_n^{n+}/PP_i^{4-} adducts may be calculated from the ΔpK_a values of protonated amines determined in excess PP_i^{4-}. As discussed above, interactions of PP_i^{4-} with neutral amines may be neglected. When 1:1 adduct formation is assumed, the apparent formation constant, $\beta_{app.}$ (AH_n^{n+}), for protonated amine, AH_n^{n+}, in the presence of PP_i^{4-} may be expressed by eqn. (2). Interactions of AH_n^{n+} with HPP_i^{3-}, $H_2PP_i^{2-}$, and

$$\beta_{app}(AH_n^{n+}) = \frac{[AH_n^{n+}] + [AH_nPP_i^{(4-n)-}]}{[A]\,[H^+]^n} \quad (2)$$

$H_3PP_i^-$ have been neglected. The mole fractions of these species are very low at pH $\approx pK_a(AH_n^{n+})$, and additionally, these species are expected to form less stable adducts than PP_i^{4-}. Substitution of eqn. (3) into eqn. (2) gives eqn. (4), where $\beta(AH_n^{n+})$ is the formation constant of AH_n^{n+} in the

$$K(AH_nPP_i^{(4-n)-}) = \frac{[AH_nPP_i^{(4-n)-}]}{[AH_n^{n+}]\,[PP_i^{4-}]} \quad (3)$$

$$\beta_{app}(AH_n^{n+}) = \beta(AH_n^{n+})\,\{K(AH_nPP_i^{(4-n)-})\,[PP_i^{4-}] + 1\} \quad (4)$$

absence of PP_i^{4-}. Table 5 summarizes the values of $\Delta\log\beta(AH_n^{n+}) = \log\beta_{app.}(AH_n^{n+}) - \log\beta(AH_n^{n+})$ when a fivefold excess of PP_i^{4-} is employed. Table 6 contains the corresponding stability constants, $K(AH_nPP_i^{(4-n)-})$, calculated from the data in Table 5. The values are compatible with those obtained previously[11] with a resin-distribution method. The standard Gibbs energy for the formation of the PP_i^{4-}-adducts of fully protonated spermidine and spermine are −15.4 and −20.0 kJ mol^{-1}, respectively. Combined with the results from calorimetric measurements, values of 120 J K^{-1} mol^{-1} (spermidine) and 160 J K^{-1} mol^{-1} (spermine) are obtained for the entropy of interaction. The thermodynamics of the adduct formation seem to exhibit features known to be typical for ion association:[24] the enthalpy change is unfavourable for adduct formation, but the relatively large positive entropy change causes the $\Delta G^\ominus$ values to be negative.

Table 5. $\Delta\log\beta(AH_n^{n+})$ values for the conjugate acids of organic amines in a fivefold excess (0.05 ml dm^{-3}) of pyrophosphate ion.[a]

A	*I*/mol dm^{-3}	$\Delta\log\beta(AH_4^{4+})$	$\Delta\log\beta(AH_3^{3+})$	$\Delta\log\beta(AH_2^{2+})$
1	0.50		1.1	0.4
	1.00		1.3	0.2
2	0.50	2.0	0.9	0
	1.00	2.1	0.7	0
3	0.50			1.1
	1.00			1.2
4	0.50			0.8
	1.00			0.7
5	0.50			0.6
	1.00			0.5

[a]Difference between the logarithmic formation constant of the protonated species in the presence and in the absence of pyrophosphate ion. $\log\beta(AH^+) < 0.2$.

Experimental

Materials. Spermidine trihydrochloride, spermine tetrahydrochloride, propane-1,3-diamine, butane-1,4-diamine, pentane-1,5-diamine and tetrasodium pyrophosphate were all commercial products from Sigma. Tetramethylammonium chloride was a product supplied by Fluka. All compounds were used as received. Solutions of tetramethylammonium hydroxide were prepared by dilution of a commercial stock solution (40%, Aldrich). The concentrations were determined with a hydrogen chloride solution prepared from standard ampoules (Baker).

Potentiometric titrations. Glass and Ag/AgCl electrodes from Radelkis were used in the potentiometric titrations. Carbon dioxide was excluded by maintaining a stream of nitrogen through the solutions. The observed emf values were transformed into hydrogen-ion concentrations by means of eqn. (5), in which j_H and j_{OH} are fitting param-

$$E = E^\circ + C\log[H^+] + j_H[H^+] + j_{OH}(K_w/[H^+]) \quad (5)$$

Table 6. Potentiometrically obtained stability constants for the 1:1 adducts of protonated amines with pyrophosphate tetraanion.

A	*I*/mol dm^{-3}	$\log K(AH_4PP_i)$	$\log K(AH_3PP_i^-)$	$\log K(AH_2PP_i^{2-})$
1	0.50		2.5	1.6
	1.0		2.7	1.2
2	0.50	3.4	2.2	
	1.0	3.5	2.0	
3	0.50			2.5
	1.0			2.6
4	0.50			2.1
	1.0			2.0
5	0.50			1.9
	1.0			1.7

eters, adjusted with the aid of calibration titrations of aqueous hydrogen chloride with tetramethylammonium hydroxide. E_0 and C are the constants of the Nernst equation, while K_w is the ionic product of water under the experimental conditions.[25] A Gauss–Newton fitting method applied to calculate the dependence of E on $[H^+]$. The successive acidity constants were obtained by the Bjerrum complex formation function using the Davidson–Fletcher–Powell minimization method.[26] The pK_a values reported are mean values of 3–5 experiments. The reproducibility was better than 0.05 for $pK < 10$ and better than 0.1 above this limit.

Calorimetric measurements. The calorimetric measurements were performed with a LKB 10700-2 Batch Microcalorimeter. The compartments of the reaction cell were loaded with aqueous solutions of tetrasodium pyrophosphate and spermidine trihydrochloride or spermine tetrahydrochloride. When the enthalpies of dilution were measured one of the solutions was replaced with distilled water. The compartments of the reference cell were filled with distilled water. The enthalpies of interaction between protonated polyamine and the pyrophosphate tetraanion were obtained by subtracting the enthalpies of dilution of the reactants from the enthalpy of mixing. No background electrolyte was used in the measurments.

References

1. Josse, J. and Wong, S. C. K. *Enzymes,* 3rd ed. *4* (1971) 499.
2. Rapoport, T. A., Höhne, W. E., Reich, J. G., Heitmann, P., Rapoport, S. M. *Eur. J. Biochem. 26* (1972) 237.
3. Unemoto, T., Tanaka, M. and Hyashi, M. *Biochim. Biophys. Acta 327* (1973) 490.
4. Schreier, E. and Höhne, W. E. *FEBS Lett. 90* (1978) 93.
5. Randhal, H. *Eur. J. Biochem. 102* (1979) 251.
6. Terzyan, S. S., Voronova, A. A., Smirnova, E. A., Kuranova, I. P., Nekrasov, Y. V., Arutyunyan, E. G., Vainstein, B. K., Höhne, W. and Hansen, G. *Biorg. Khim. 10* (1984) 1469.
7. Lahti, R. and Jokinen, M. *Biochemistry 24* (1985) 3526.
8. Gonzales, M. A. and Cooperman, B. S. *Biochemistry 25* (1986) 7179.
9. Lahti, R. *Microbiol. Rev. 47* (1983) 169.
10. Lahti, R. and Lönnberg, H. *Biochem. J. 231* (1985) 485.
11. Lahti, R., Hannukainen, R. and Lönnberg, H. *Biochem. J. 259* (1989) 55.
12. Jenkins, T. *Anal. Biochem. 145* (1985) 362.
13. Palmer, B. N. and Powell, H. K. J. *J. Chem. Soc., Dalton Trans.* (1974) 2089.
14. Kimberley, M. M. and Goldstein, J. H. *Anal. Chem. 53* (1981) 789.
15. Takeda, Y., Samejima, K., Nagano, K., Watanabe, M., Sugeta, H. and Kyogolu, Y. *Eur. J. Biochem. 130* (1983) 383.
16. Templeton, D. M. and Sarkar, B. *Can. J. Chem. 63* (1985) 3122.
17. Sigel, H., Huber, P. R. and Pasternack, R. F. *Inorg. Chem. 10* (1971) 2226.
18. Schwarzenbach, G., Maissen, B. and Ackermann, H. *Helv. Chim. Acta 35* (1952) 2333.
19. McNabb, W. W., Hazel, J. F. and Baxter, R. A. *J. Inorg. Nucl. Chem. 30* (1968) 1585.
20. Irani, R. R. *J. Phys. Chem. 65* (1961) 1463.
21. Lambert, S. M. and Watters, J. I. *J. Am. Chem. Soc. 79* (1957) 4262.
22. Aikens, D. A., Bunce, S. C., Oansch, O. F., Schwartz, H. M. and Hurwitz, C. *J. Chem. Soc., Chem. Commun.* (1983) 43; *Biophys. Chem. 17* (1983) 67; *Biophys. Chem. 19* (1984) 245.
23. Delfini, M., Segre, A. L., Conti, F., Barbucci, R., Barone, V. and Ferruti, P. *J. Chem. Soc., Perkin Trans. 2* (1980) 900.
24. Gordon, J. E. *The Organic Chemistry of Electrolyte Solutions,* Wiley, New York 1975, pp. 405–409.
25. Högfeldt, E. *Stability Constants of Metal-Ion Complexes. Part A: Inorganic Ligands,* Pergamon Press, Oxford 1982.
26. Himmelblau, D. M. *Applied Nonlinear Programming,* McGraw-Hill, New York 1972.

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