N,N',N'',N'''-(2-Aminoethyl)-1,4,8,11-tetraazacyclotetradecane (TAEC) as a Polyammonium Receptor for Anions

Andrea Bencini,[a] Antonio Bianchi,*[,a] Maria I. Burguete,[b] Antonio Domenech,[c] Enrique García-España,*[,c] Santiago V. Luis,*[,b] Miguel A. Niño[b] and José A. Ramírez[c]
[a] *Department of Chemistry, University of Florence, Via Maragliano 75/77 I-50144 Firenze, Italy*
[b] *Department of Organic Chemistry, University of Valencia, Colegio Universitario de Castellón, 12080 Castellón, Spain*
[c] *Department of Inorganic Chemistry, University of Valencia, c/o Dr. Moliner 50, 46100 Burjassot, Valencia, Spain*

The protonation behaviour of the polyammonium receptor *N,N',N'',N'''*-(2-Aminoethyl)-1,4,8,11-tetraazacyclotetradecane (TAEC) and its interaction with ATP, $[Fe(CN)_6]^{4-}$, $[Co(CN)_6]^{3-}$ and $P_2O_7^{4-}$ have been studied potentiometrically in 0.15 mol dm^{-3} $NaClO_4$ at 298.15 K as well as by ^1H, ^{13}C and ^{31}P NMR and electrochemical techniques. Emf data analysis indicates 1:1 stoichiometries for all the adducts formed. In the case of ATP and $[Fe(CN)_6]^{4-}$ the stoichiometries are confirmed by using ^{31}P NMR and electrochemical measurements, respectively. The results are compared with those of the related macrocyclic polyamine [24]aneN_8 and discussed in terms of the different ligand topologies.

Over the last two decades, much attention has been devoted to the study of anion co-ordination chemistry.[1–4] Polyammonium entities have been revealed to be good co-ordinating agents for anions. Some of the main characteristics of their chemistry making this species suitable for developing anion co-ordination are: (i) high water solubility, (ii) ability to form highly charged species at neutral pH and (iii) possibility of formation of hydrogen-bond networks.

It is well known that some biogenic polyamines like spermine and spermidine in their protonated forms interact with nucleotide and nucleic acids.[5] Accordingly, a number of polyamines have been synthesized and studied as suitable receptors for biologically significant anions, such as ATP, ADP and polyphosphates, as well as other phosphate-type anions.[6–10]

Studies with macrocyclic and linear polyamines have been reported. In some cases, it has been found that the interaction ATP–polyammonium receptor is accompanied by an increase in the rate of ATP hydrolysis, where the polyamine acts as a catalyst.[7,11–16] The topology of the polyammonium receptor seems to play an essential role in the magnitude of the catalytic effect.

However, until now no studies have been reported on the interaction of ATP with highly branched polyazacycloalkanes containing primary amino groups in the arms. *N,N',N'',N'''*-(2-Aminoethyl)-1,4,8,11-tetraazacyclotetradecane (hereafter TAEC) is characteristic of this class of ligands. Despite its promising topology (see ligand drawing), this ligand has been used only for the preparation of crystalline binuclear copper(II), nickel(II) and cobalt(II) complexes.[17] In the present paper we report on the protonation behaviour of TAEC and on its interaction with ATP and $P_2O_7^{4-}$ as well as the complex anions $[Co(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$. In order to understand the disposition of the polyammonium sites, the protonation pattern of TAEC has also been analysed by using ^1H and ^{13}C NMR spectroscopy.

Results and Discussion

The ligand TAEC was synthesized following the procedure described by Kida *et al.*[17] Cyclam (1,4,8,11-tetraazacyclotetradecane) reacts in good yield with *N*-tosylaziridine (4 mol) to give 1,4,8,11-tetrakis-(*p*-tolylsulphonyl)-1,4,8,11-tetraazacyclotetradecane. In our case, detosylation was best carried out by use of 96% H_2SO_4.[18] The sulphate was transformed into the free base by treatment with NaOH or, better, by using an anion-exchange resin in its basic form. Finally, the amine was purified and kept as its octahydrobromide salt.

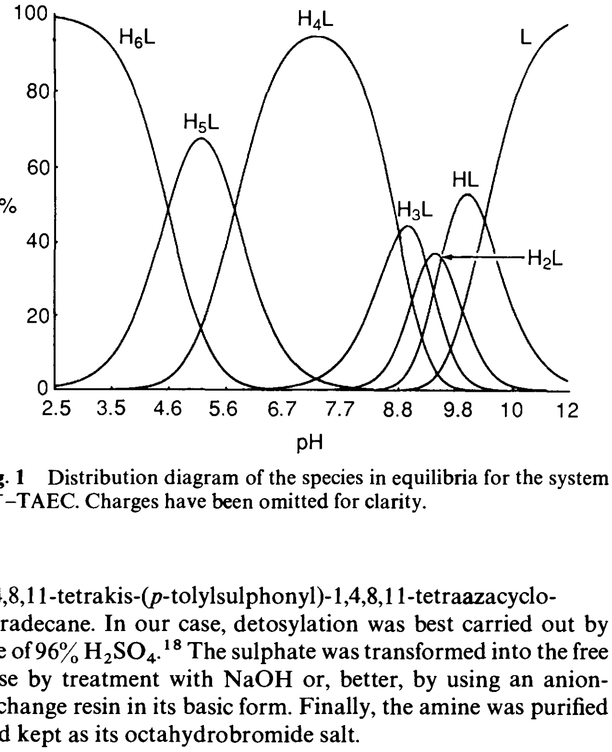


Fig. 1 Distribution diagram of the species in equilibria for the system H^+–TAEC. Charges have been omitted for clarity.

In Table 1 the basicity constants of TAEC together with those of the related cyclic and open-chain ligands with eight nitrogen donors, 1,4,7,10,13,16,19,22-octaazacyclotetracosane ([24]aneN_8), 1,22-dimethyl-1,4,7,10,13,16,19,22-octaazadocosane (Me_2heptaen), are presented. For the sake of comparison, the protonation constants of the ligand *N,N',N'',N'''*-tetramethyltetraazacyclotetradecane (Me_4cyclam) have also been included in Table 1.[19–21] In Fig. 1 the distribution diagram for the species existing in equilibria between pH 2 and 11 is presented. It can be observed that the protonation constants of TAEC are distributed in two groups; TAEC behaves as a strong base in its first four protonation steps and as a much weaker base in the last ones. As a matter of fact, it has not been possible to determine the last two protonation constants in aqueous solution. On the basis of available data, it can be proposed that

Table 1 Protonation constants of the ligands TAEC, [24]aneN_8, Me_2heptaen and Me_4cyclam

Reaction	TAEC[a]	[24]aneN_8[a,b]	Me_4cyclam[c]	Me_2heptaen[d]
$L + H = HL$	10.41(1)[e]	9.65	9.70	10.39
$L + 2H = H_2L$	20.04(2)			
$L + 3H = H_3L$	29.39(2)			
$L + 4H = H_4L$	38.22(2)			
$L + 5H = H_5L$	44.03(3)			
$L + 6H = H_6L$	48.61(2)			
$L + 7H = H_7L$	50.2(2)			
$H + HL = H_2L$	9.63	9.33	9.31	9.77
$H + H_2L = H_3L$	9.35	8.76	3.09	9.28
$H + H_3L = H_4L$	8.83	7.87	2.64	8.61
$H + H_4L = H_5L$	5.81	4.55		6.68
$H + H_5L = H_6L$	4.58	3.42		4.44
$H + H_6L = H_7L$		2.71		2.31
$H + H_7L = H_8L$		1.8		2.93

[a] Constants determined in 0.15 mol dm^{-3} $NaClO_4$ at 298 K. [b] From ref. 19. [c] From ref. 21. [d] From ref. 20. [e] Values in parentheses are the standard deviation in the last significant figure.

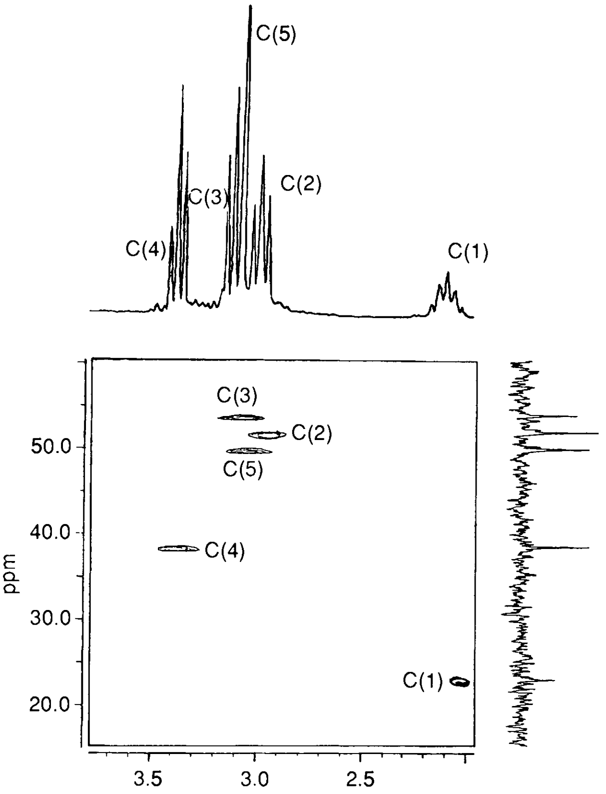


Fig. 2 ^1H–^{13}C HETCOR 2D spectra of TAEC recorded at pH 9

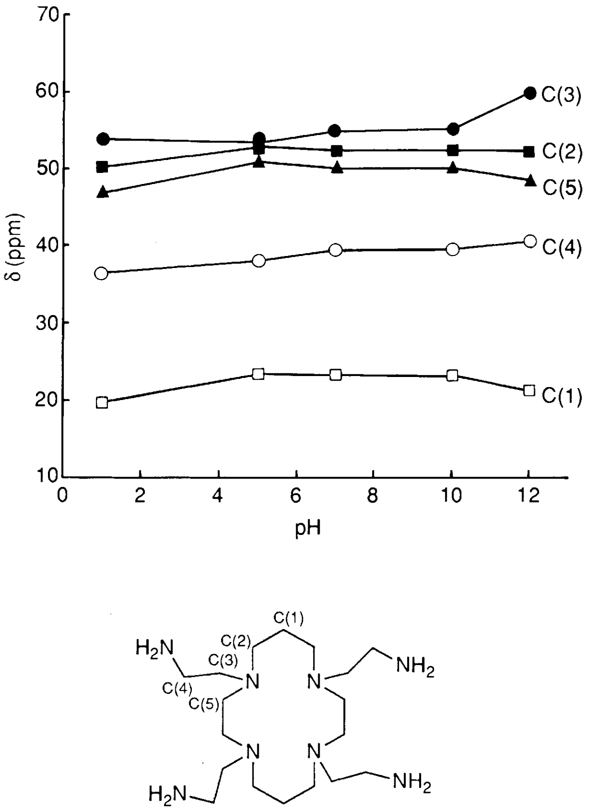


Fig. 3 Plot of the ^{13}C NMR chemical shifts of the ligand TAEC *vs.* pH

protons attach first to the primary amino groups of the arms and secondly to the tertiary nitrogens of the macrocycle.

^1H and ^{13}C NMR spectra of TAEC recorded at different pH values fully agree with this assumption. The assignment of all the ^1H and ^{13}C signals at the different pH values has been confirmed by using selective decoupling and ^1H–^{13}C HETCOR 2D experiments (see Fig. 2). In Fig. 3 the variations of the ^{13}C chemical shifts with pH are presented. The ^{13}C spectrum for TAEC recorded at very basic pH consists of five signals at 21.3 [C(1)], 40.9 [C(4)], 48.7 [C(5)], 52.5 [C(2)] and 60.2 [C(3)] which exhibit significant variations as the pH is decreased. As shown in the distribution diagram (Fig. 1), when pH goes from basic to neutral, TAEC reaches a maximum protonation degree of 4. Within this pH range, the carbon atoms C(3) and C(4) are the only ones that experience upfield shifts. Therefore, the first four protonation steps should involve the primary amino groups of the arms. This shift is greater for C(3) than for C(4), in agreement with the β-shift reported for this kind of compound.[22] A downfield shift is observed for the macrocyclic carbon atoms [C(1), C(2) and C(5)], and only when the pH value is lowered below 7, is an upfield shift experienced by these macrocyclic carbon atoms. All of these facts support the initial assumption, based on the thermodynamic data, that the protonation of the macrocyclic nitrogens takes place only below pH 7.

The ^1H NMR spectra also agree with these considerations. At pH 7, where TAEC is in its tetraprotonated form, $[H_4TAEC]^{4+}$, the ^1H NMR spectrum consists of an A_2B_2 spin system for the protons of the ethylenic groups in the arms [C(3) and C(4)]; at pH 3, where TAEC is in its hexaprotonated form, $[H_6TAEC]^{6+}$, the signals for the same protons consist of a complex set of peaks characteristic of an AA′BB′ pattern with a corresponding loss of magnetic equivalence. The other

Table 2 Logarithms of the stability constants for the interaction of TAEC with the anions ATP^{4-}, $[Fe(CN)_6]^{4-}$, $[Co(CN)_6]^{3-}$ and $P_2O_7^{4-}$ in 0.15 mol dm^{-3} $NaClO_4$ at 298 K[a]

	$A = ATP^{4-}$		$[Fe(CN)_6]^{4-}$	$[Co(CN)_6]^{3-}$	$P_2O_7^{4-}$	
Reaction	TAEC	[24]aneN_8	TAEC	TAEC	TAEC	[24]aneN_8
L + 3 H + A	31.0(1)[b]					
L + 4 H + A	40.43(3)		40.12(8)			
L + 5 H + A	47.96(2)		47.52(2)	46.0(2)		
L + 6 H + A	54.29(1)		53.47(1)	51.39(5)	55.15(6)	
L + 7 H + A	58.93(1)			53.55(7)	61.04(2)	
L + 8 H + A	62.64(1)					
L + 9 H + A	64.04(4)					
H_3L + A	1.7					
H_4L + A	2.18	3.74[c]	1.8			
H_5L + A	3.87	6.93	3.39	2.03		
H_6L + A	5.71	9.79	4.73	2.78	6.41	11.56[c]
H_7L + A	8.11	11.57				

[a] Some of the constants for the interaction of [24]aneN_8 with the same anions under the same conditions are also included. [b] Values in parentheses are standard deviations in the last significant figure. [c] From ref. 15.

hydrogens of TAEC, although exhibiting significant variations in their chemical shifts, do not show any changes in their spin systems over all the pH range studied, the protons of C(5) remaining as a singlet, those of C(2) as a triplet and those of C(1) as a quintuplet.

The branched macrocycle TAEC can be regarded as an assembly of four ethylenediamine moieties with the tetraaza macrocycle cyclam. The first protonation constant of TAEC is higher than the corresponding one for ethylenediamine (log $K_1 = 9.67$),[23] the statistical factor accounting for this difference.[24] In TAEC there are four equivalent positions to which the first proton can be attached, while in ethylenediamine there are only two. Accordingly, a difference of *ca.* 0.69 logarithm units is to be expected between the values of log K_1 for both compounds (experimental difference 0.74). This would suggest that intramolecular hydrogen bonding between the arms is not an additional stabilization factor in the protonation of TAEC. Note the similarity between the first four basicity constants of TAEC and those of the related open-chain polyamine 1,22-dimethyl-1,4,7,10,13,16,19,22-octaazadocosane (Me_2heptaen) (see Table 1). These basicity constants of TAEC are, on the other hand, higher than those of the cyclic polyamine 1,4,7,10,13,16,19,22-octaazacyclotetracosane ([24]-aneN_8), suggesting, for the same degree of protonation, a greater density of positive charge in the macrocycle. The last two constants (log $K_5 = 5.84$ and log $K_6 = 4.61$), which involve the macrocyclic nitrogens, are less basic than the first two of the related tetraazamacrocycle *N,N′,N″,N‴*-tetramethyltetraazacyclotetradecane (Me_4cyclam)[21] (see Table 1). This fact can be attributed to the higher positive charge present in the tetraprotonated TAEC. As already indicated, TAEC at neutral pH is mainly in its tetraprotonated form (see Fig. 1), suggesting that TAEC could be a potential receptor for anions at pH values of biological significance. On the other hand, because of the initial protonation in the arms, the flexibility of this receptor might lead to better matching in its interaction with the guests.

Interaction with Anions.—In Table 2 the stability constants related to the interaction of TAEC with ATP, as well as with the tri- and tetra-charged anions $[Fe(CN)_6]^{4-}$, $P_2O_7^{4-}$ and $[Co(CN)_6]^{3-}$, are reported. All these constants have been determined potentiometrically in 0.15 mol dm^{-3} $NaClO_4$ solution at 298.15 K. Some of the constants previously reported for the interaction of these anions with the macrocycle [24]aneN_8 have also been included in Table 2.

Analysis of the emf data with the computer program SUPERQUAD[25] gives predominant 1:1 receptor:anion stoichiometries for all the species formed. In the case of ATP, we have also followed its interaction with TAEC by means of ^{31}P NMR spectroscopy. As shown in Fig. 4(*a*), the ^{31}P NMR spectra of ATP at pH 6 exhibit successive changes with the addition of TAEC. Note that the NMR signals of the ATP γ and β P atoms are shifted upfield with the addition of TAEC while the signal of the α phosphorus remains almost unaffected. P_γ is most sensitive to the addition of TAEC as usually observed for other polyammonium receptors. As shown in Fig. 4, for ratios [TAEC]:[ATP] > 1, no further changes in the chemical shifts of the phosphorus atoms are produced. A plot of the variation in the ^{31}P chemical shifts as a function of the [TAEC]:[ATP] ratio [Fig. 4(*b*)] indicates, in agreement with the emf data analysis, 1:1 stoichiometries for the adducts ATP:TAEC.

From the values of the stability constants (Table 2), the following observations can be made. For a 1:1 [TAEC]:[ATP] ratio (see distribution diagram, Fig. 5), the interaction results are appreciable from pH values of *ca.* 9.5. As already observed in other polyammonium–ATP systems, the strength of the interaction increases with the charge on the receptor. In our case, the constants vary from log $K = 1.7$, for $H_3TAEC^{3+} + ATP^{4-} \rightleftharpoons [H_3(TAEC)ATP]^-$, to log $K = 5.71$, for $H_6TAEC^{6+} + ATP^{4-} \rightleftharpoons [H_6(TAEC)ATP]^{2+}$. However, as TAEC can take up a maximum of six protons in solution, at least until pH 2 (see Fig. 1), the seventh protonation in the adduct, which takes place below pH 2, should, in principle, affect ATP instead of TAEC. As a matter of fact, the ^{31}P chemical shifts of the ATP P_γ atom in the presence of one equivalent of TAEC at pH values below 4, do not vary significantly compared with those of free ATP at the same pH, which confirms this assumption. Therefore, a maximum interaction between ATP and TAEC occurs at pH values around 6, where the species $[H_6(TAEC)ATP]^{6+}$ predominates in solution (see Fig. 5). Accordingly, the maximum variation between the ^{31}P chemical shifts of P_γ for ATP free and in the presence of one equivalent of TAEC is observed at this pH. The following stepwise constants should, therefore, be considered as corresponding to the addition of protonated forms of ATP to the hexaprotonated TAEC. Another feature to be considered is the smaller interaction between TAEC and ATP compared with that displayed by the related macrocycle [24]aneN_8. For this macrocyclic ligand, the stability constants are, for all the protonation degrees, higher than the corresponding ones for TAEC. The difference between the log K values for the ATP complexes of both ligands, $[(ATP)H_nL]^{n-4}$, increases with the protonation degree, thus

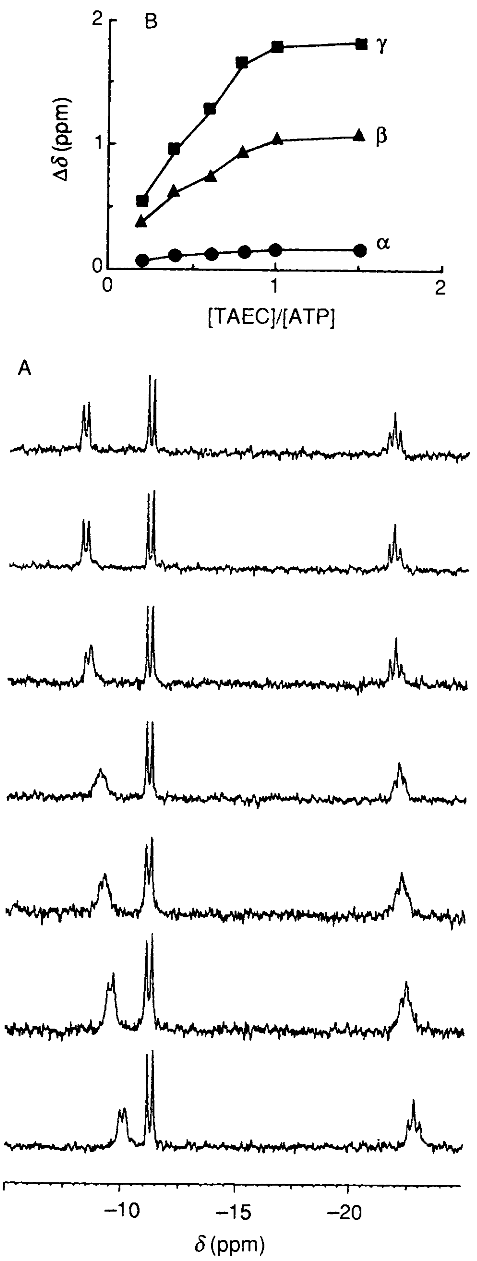


Fig. 4 A, ^{31}P NMR spectra of ATP as a function of increasing *R*(= [TAEC]/[ATP]) at pH 6. *R* = 0(*a*), 0.2(*b*), 0.4(*c*), 0.6(*d*), 0.8(*e*), 1.0(*f*) and 1.5(*g*). B, ^{31}P NMR shifts (|Δδ|) of the α, β and γ-phosphate signals of ATP as a function of increasing [TAEC]:[ATP] ratio at pH 6 (ATP = 0.01 mol dm^{-3}).

reflecting a greater accumulation of positive charge in the macrocyclic structure of [24]aneN$_8$, as was also suggested by the protonation study.

Concerning the ATP dephosphorylation induced by polyammonium cations, it has been stated that although the interaction between receptor and substrate is required as a first step in the catalytic pathway, the main factors determining the catalytic activity are the complementarity between the structures of substrate and receptor, and the presence of well disposed nucleophilic nitrogens in the receptor.[26] In our case, despite the, at first glance, promising structure of TAEC, a low catalytic effect has been observed. In Fig. 6, variation of the ^{31}P NMR spectra of 1:1 ATP:TAEC mixtures with time is presented. The half-life for the hydrolytic reaction of ATP in the presence of [24]aneN$_8$ is several orders of magnitude shorter than that in the presence of TAEC.[15]

In order to ascertain whether this small interaction of TAEC with ATP is also maintained for other anions, we have extended our potentiometric studies to the interaction of TAEC with the tri- and tetra-charged anions $P_2O_7^{4-}$, $[Co(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$. As observed in Table 2, the interaction is higher for the tetravalent anions $P_2O_7^{4-}$ and $[Fe(CN)_6]^{4-}$ than for $[Co(CN)_6]^{3-}$. If these results are compared with those of [24]aneN$_8$ (Table 2), it can be concluded that TAEC has a lower tendency to co-ordinate anions. For $[H_6(A)TAEC]^{2+}$, the species where A = $P_2O_7^{4-}$ has a higher formation constant

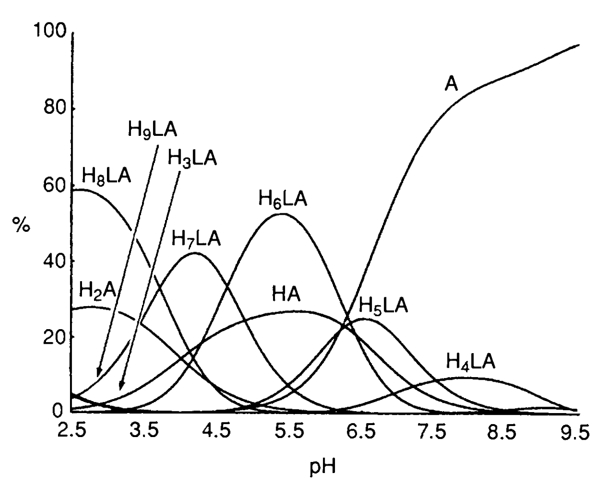


Fig. 5 Distribution diagram for the species existing in equilibria in the system ATP–TAEC. [TAEC] = [ATP] = 10^{-3} mol dm^{-3}, A = ATP. Charges have been omitted.

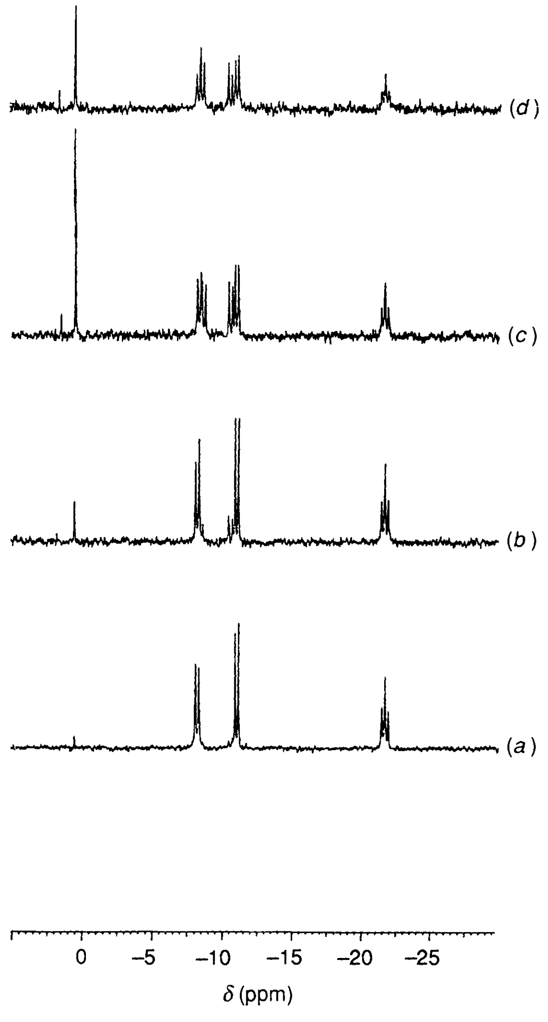


Fig. 6 Time dependence of the ^{31}P NMR spectrum of TAEC/ATP mixtures. *t*/h = 1(*a*), 7(*b*), 30(*c*), (*d*). *R* = 1(*a*), (*b*), (*c*), 1.5(*d*).

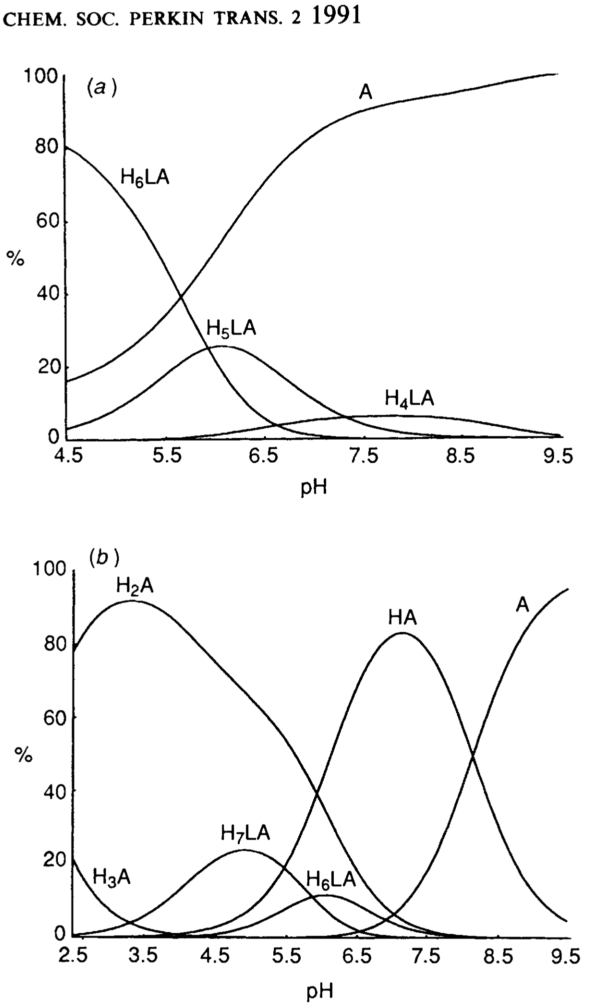


Fig. 7 (*a*) Distribution diagram for the species existing in equilibria in the system $[Fe(CN)_6]^{4-}$–TAEC. $[[Fe(CN)_6]^{4-}] = [TAEC] = 10^{-3}$ mol dm^{-3}, A = $[Fe(CN)_6]^{4-}$. Charges have been omitted. (*b*) Distribution diagram for the species existing in equilibria in the system $P_2O_7^{4-}$–TAEC.[TAEC] = $[P_2O_7^{4-}] = 10^{-3}$moldm^{-3},A = $P_2O_7^{4-}$. Charges have been omitted.

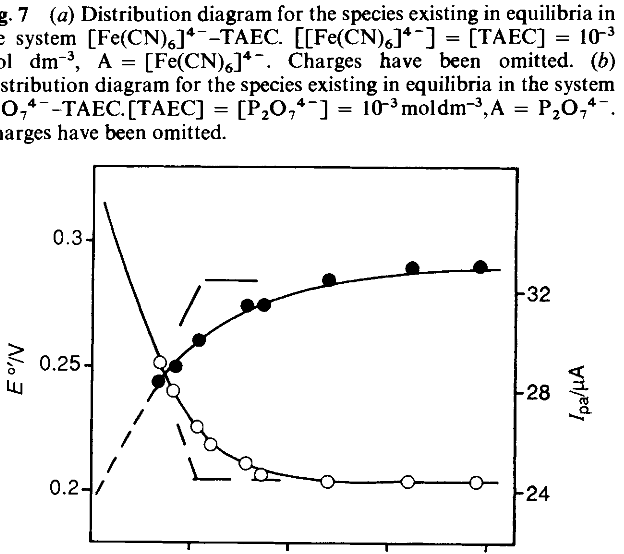


Fig. 8 Plot of the formal potential and peak current *vs.* the ratio [TAEC]:$[Fe(CN)_6]^{4-}$] at pH = 4.5. $[[Fe(CN)_6]^{4-}] = 10^{-3}$ mol dm^{-3}. Platinum electrode, r = 0.09 V s^{-1}.

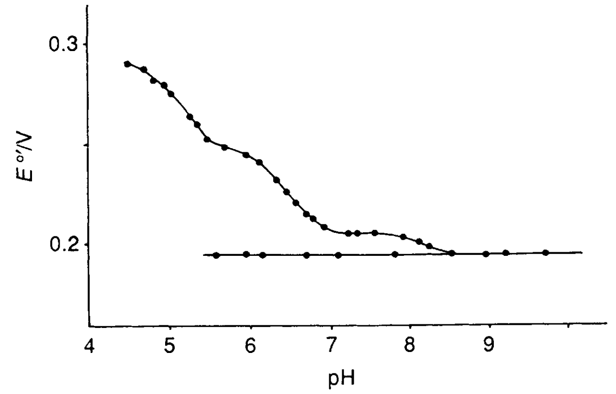


Fig. 9 pH dependence of formal potentials estimated from the CV recorded for the $[Fe(CN)_6]^{4-}$–TAEC solutions. Platinum working electrode, sweep rate: 100 mV s^{-1}. $[[Fe(CN)_6]^{4-}] = 10^{-3}$ mol dm^{-3}, [TAEC] = 3.5 × 10^{-3} mol dm^{-3}.

than that for A = $[Fe(CN)_6]^{4-}$. However, the distribution diagram for the system $P_2O_7^{4-}$–TAEC calculated for the 1:1 molar ratio, shows a very low percentage of formation of this species, while in the corresponding distribution diagram for the system $[Fe(CN)_6]^{4-}$–TAEC the percentages of formation of the adducts are much higher (see Fig. 7). At the pH values where the $P_2O_7^{4-}$–TAEC complexes are formed, owing to the relatively high basicity of $P_2O_7^{4-}$, the amount of $P_2O_7^{4-}$ present in solution in the form of the tetravalent anion is negligible.

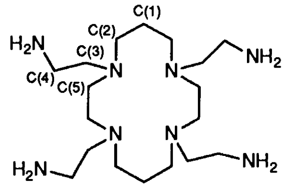
We have also applied electrochemical methods to study the $[Fe(CN)_6]^{4-}$–TAEC system. Cyclic voltammograms of hexacyanoferrate(II) solutions exhibit a well known reversible one-electron transfer. This electrochemical pattern is altered by adding small amounts of TAEC, confirming the formation of complex species. At a given pH, the voltammograms recorded for small values of the ligand to metal ratio are rather broad and look like the superposition of two waves. For ligand-to-metal ratios close to unity, the shape of the cyclic voltammograms remains almost unchanged and is that expected for a reversible diffusion-controlled one-electron transfer. Accordingly, plots of peak potential and peak current against the ligand-to-metal ratio show a sharp break close to 1 (see Fig. 8). This indicates that complexes with 1:1 hexacyanoferrate(II)–TAEC stoichiometry are formed, as confirmed by comparing experimental current–potential curves with theoretical voltammograms.[27]

CV–pH titrations made for ligand-to-metal ratios between 1.5 and 3.5 confirm the stepwise pH-dependent formation of different complex species. As shown in Fig. 9, the variation with pH of the redox potential [$E^{o\prime} = (E_{pa} + E_{pc})/2$] of the couple $Fe^{II}LHp \rightleftharpoons Fe^{III}LHp + e^-$ closely agrees with the distribution diagram deduced from the potentiometric measurements [Fig. 7(*a*)]. The stability constant for the hexacyanoferrate(III) species, $[H_6(TAEC)Fe(CN)_6]^{3+}$, was determined from the break or plateau values of the shift in the formal potential with respect to the formal potential of the $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ couple and the stability constant previously determined by potentiometry for the hexacyanoferrate(II) species.[28] The value obtained for the stepwise stability constant of the reaction, $[H_6(TAEC)]^{6+} + [Fe(CN)_6]^{3-} \rightleftharpoons [H_6(TAEC)Fe(CN)_6]^{3+}$ (log K = 2.66), is very close to that obtained potentiometrically for the similar species containing the complex anion $[Co(CN)_6]^{3-}$ (log K = 2.78). This is to be expected, because of the similar charge and topology of $[Co(CN)_6]^{3-}$ and $[Fe(CN)_6]^{3-}$ species.

Experimental

Preparation of the Ligand.—The ligand was synthesized following the procedure described by Kida *et al.*[17] with some differences. The complete synthesis and the spectroscopic characterization of the different compounds are described.

N,N′,N″,N‴-*Tetrakis*(2-*tosylaminoethyl*)-1,4,8,11-*tetraazacyclotetradecane*. This compound was obtained as described in ref. 17: m.p. 210–211 °C; ν_{max}(KBr)/cm^{-1} 3260 (NH), 1340



(SO_2) and 730 (SN); δ_H($CDCl_3$; 200 MHz) 7.7 (8 H, d), 7.2 (8 H, d), 3.0 (8 H, m), 2.4 (32 H, m) and 2.2 (8 H, m).

N,N′,N″,N‴-*Tetrakis*(*2-aminoethyl*)-1,4,8,11-*tetraazacyclotetradecane* (*TAEC*). The previous compound (1.5 g, 1.56 mmol) was dissolved in 96% H_2SO_4 and heated to 110 °C for 65 h. The solution was cooled in an ice bath and diethyl ether was added dropwise until a crystalline grey solid was formed. The solid was washed with diethyl ether under an argon atmosphere and then dissolved in a small amount of water and passed through an Amberlite IRA-400 ion-exchange resin in its basic form. The solution was vacuum evaporated to dryness to yield the product as a yellow oil. This oil was dissolved in ethanol, the solution cooled in an ice bath and the stoichiometric amount of a concentrated HBr solution was added. With time, the trihydrate of the salt was obtained as a white solid (1.38 g, 86%), m.p. 250 °C (decomp.). IR (KBr) ν_{max}/cm^{-3} 3420 (N–H), 2640 (N^+–H), 1460 (C–N). δ_H(D_2O, 200 MHz) 3.5–2.75 (32 H, m), 2.1–1.8 (4 H, m). δ_C(D_2O, 50.3 MHz) 53.49, 53.06, 51.06, 38.13, 23.57 (Found: C, 21.0; H, 5.2; N, 11.2. Calc. for $C_{18}H_{58}Br_8N_8O_3$: C, 21.2; H, 5.15; N, 11.0%).

EMF Measurements.—Potentiometric measurements were carried out by using equipment (potentiometer, burette, cell, stirrer, microcomputer, *etc.*) that has been described previously.[29] The reference electrode was Ag/AgCl in saturated KCl solution. The glass electrode (Orion model 91-01) was calibrated as a hydrogen-concentration probe by titration of known amounts of HCl with CO_2-free NaOH solutions and determining the equivalent point by the Gran method,[30] which provides the standard potential of the cell and the ionic product of water [$pK_w = 13.73(1)$ at 298.15 K in 0.15 mol dm^{-3} $NaClO_4$]. The protonation constants of ATP^{4-} and $P_2O_7^{4-}$ were taken from ref. 15. The program SUPERQUAD[25] was used to calculate the equilibrium constants. The titration curves for each system were treated as a single set or separately without causing significant variations in the values of the constants. Furthermore, the sets of data were merged together and treated simultaneously to give the final stability constants. The $K_3[Co(CN)_6]$ used in the potentiometric measurements was purchased from Aldrich and recrystallized twice from ethanol–water mixtures.

NMR Spectroscopy.—^1H and ^{13}C NMR spectra were recorded at 200.0 and 50.32 MHz on a Varian Gemini 200 instrument using either Me_4Si or DSS* as the internal reference. ^{31}P NMR spectra were recorded at 80.015 MHz in a Bruker AC-200 spectrometer using 85% H_3PO_4 as an external reference.

Electrochemical Measurements.—Cyclic voltammograms were obtained using gold, platinum and glassy carbon working electrodes with the sweep rate varied from 50–500 mV s^{-1}. The measurements were made *vs.* a saturated calomel reference electrode (SCE) in a standard three-electrode cell with a Pt-wire auxiliary electrode. Electrochemical data were obtained with a signal generator Newtronics 200P, a potentiostat AR and an *x*–*y* recorder Riken-Denshi F35. All experiments were made in 0.15 mol dm^{-3} $NaClO_4$ as the supporting electrolyte under an argon atmosphere at 298 K.

To obtain the molar ratio saturation curves a series of samples containing a constant amount of potassium ferrocyanide and varying concentrations of TAEC were prepared, adjusting the pH to the required value by adding appropriate amounts of NaOH and/or $HClO_4$ solutions.

For the pH–CV titration, alkaline solutions with a given ligand-to-metal ratio were prepared. In each experiment $HClO_4$ was added in stages in order to vary the pH between 10 and 4.

Acknowledgements

We thank the Institut Valencià d'Estudis i Investigació for financial support to this work. We are also indebted to the Italian Ministero dell'Università e della Ricerca Scientifica e Tecnologica.

References

1 (*a*) F. Vögtle, H. Sieger and W. H. Müller, *Top. Curr. Chem.*, 1982, **98**, 107; (*b*) E. Kimura, *Top. Curr. Chem.*, 1985, **128**, 113.
2 H. M. Colquhoun, J. F. Stoddart and D. J. Williams, *Angew. Chem., Int. Ed. Engl.*, 1986, **25**, 487.
3 P. G. Potvin and J. M. Lehn, in *Synthesis of Macrocycles*, eds. R. M. Izatt and J. J. Christensen, Wiley, New York, 1987, p. 167.
4 F. P. Schmidtchen, A. Gleich and A. Schimmer, *Pure Appl. Chem.*, 1989, **61**, 1535.
5 C. Nakai and W. Glinsman, *Biochemistry*, 1977, **16**, 5636.
6 B. Dietrich, M. W. Hosseini, J. M. Lehn and R. B. Sessions, *J. Am. Chem. Soc.*, 1981, **103**, 1282.
7 M. W. Hosseini, J. M. Lehn, L. Maggiora, K. B. Mertes and M. P. Mertes, *J. Am. Chem. Soc.*, 1987, **109**, 537.
8 M. W. Hosseini and J. M. Lehn, *Helv. Chim. Acta*, 1987, **70**, 1312.
9 J. F. Marecek and C. J. Burrows, *Tetrahedron Lett.*, 1986, **27**, 5943.
10 J. F. Marecek, C. P. A. Fischer and J. Burrows, *Tetrahedron Lett.*, 1988, **29**, 6231.
11 M. W. Hosseini, J. M. Lehn and M. P. Mertes, *Helv. Chim. Acta*, 1983, **66**, 2454.
12 P. G. Yohannes, M. P. Mertes and K. B. Mertes, *J. Am. Chem. Soc.*, 1985, **107**, 8288.
13 P. G. Yohannes, K. E. Plute, M. P. Mertes and K. B. Mertes, *Inorg. Chem.*, 1987, **26**, 1751.
14 M. W. Hosseini, J. M. Lehn, K. C. Jones, K. E. Plute, K. B. Mertes and M. P. Mertes, *J. Am. Chem. Soc.*, 1989, **111**, 6330.
15 A. Bencini, A. Bianchi, E. García-España, K. B. Mertes, M. P. Mertes and P. Paoletti, *Org. Biochem.*, in the press.
16 M. W. Hosseini, A. J. Blacker and J. M. Lehn, *J. Am. Chem. Soc.*, 1990, **112**, 3896.
17 (*a*) I. Murase, M. Mikuriya, H. Snoda and S. Kida, *J. Chem. Soc., Chem. Commun.*, 1984, 692; (*b*) I. Murase, M. Mikuriya, H. Snoda and S. Kida, *J. Chem. Soc., Dalton Trans.*, 1986, 953; (*c*) I. Murase, I. Ueda, N. Marubayashi, S. Kida, N. Matsumoto, M. Kudo, M. Toyohara, K. Hiate and M. Mikuriya, *J. Chem. Soc., Dalton Trans.*, 1990, 2763.
18 A. Bianchi, S. Mangani, M. Micheloni, V. Nanini, P. L. Orioli, P. Paoletti and B. Seghi, *Inorg. Chem.*, 1985, **24**, 1182.
19 A. Bencini, A. Bianchi, P. Dapporto, E. García-España, M. Micheloni and P. Paoletti, *Inorg. Chem.*, 1989, **28**, 1188.
20 J. Aragó, A. Bencini, A. Bianchi, E. García-España, M. Micheloni, P. Paoletti, J. A. Ramírez, P. Paoletti and P. Paoli, *Inorg. Chem.*, 1991, **30**, 1843.
21 M. Micheloni, A. Sabatini and P. Paoletti, *J. Chem. Soc., Perkin Trans. 2*, 1978, 828.
22 J. E. Sarnessky, H. L. Surprenant, F. K. Molen and C. N. Reilley, *Anal. Chem.*, 1975, **47**, 2116.
23 P. Paoletti, *Pure Appl. Chem.*, 1984, **56**, 491.
24 J. Clark and D. D. Perrin, *Q. Rev. Chem. Soc.*, 1964, **18**, 295.
25 P. Gans, A. Sabatini and A. Vacca, *J. Chem. Soc., Dalton Trans.*, 1985, 1195.
26 M. P. Mertes and K. B. Mertes, *Acc. Chem. Res.*, 1990, **23**, 413.
27 A. Domenech, unpublished work.

---

* Sodium 2,2-dimethyl-2-silapentane-5-sulphonate.

28 D. R. Crow, *Polarography of Metal Complexes*, Academic Press, London, 1960.

29 A. Bencini, A. Bianchi, M. Castelló, M. Di Vaira, J. Faus, E. García-España, M. Micheloni and P. Paoletti, *Inorg. Chem.*, 1989, **28**, 347.

30 G. Gran, *Analyst (London)*, 1952, **77**, 661.

Paper 1/00875G
Received 22*nd February* 1991
Accepted 10*th May* 1991