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Chemical speciation of organic matter in natural waters. Interaction of nucleotide 5' mono-, di- and triphosphates with major components of seawater

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ABSTRACT

The interactions of nucleotide 5' mono-, di- and triphosphates in a multicomponent ionic medium simulating the macro-composition of seawater (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , SO_4^{2-} , Synthetic Sea Water, SSW) have been investigated at different ionic strengths and at $T = 25^\circ\text{C}$. A chemical speciation model, according to which all the internal interactions between the components of the ionic medium are taken into account, was applied to determine the effective formation constants of species in the nucleotide–seawater system. The results were compared to protonation parameters calculated from single electrolyte systems. A simpler model (SSW considered as a single salt BA, with B^{z+} and A^{z-}), representative of the cation (Na^+ , K^+ , Ca^{2+} , Mg^{2+}) and anion (Cl^- , SO_4^{2-}) macro-components of seawater respectively, was also used to calculate the overall complexing ability of the seawater salt towards all the systems here investigated.

Keywords: nucleotides, complex formation, natural waters, chemical speciation of organic matter

INTRODUCTION

Nucleotides are components of nucleic acids and play a very important role in all biological systems since they are involved in most biochemical and enzymatic processes. ADP and ATP act as reservoirs and energy conveyers in terrestrial and marine photosynthetic processes. It can be affirmed that nucleotides are biologically ubiquitous substances. The occurrence of biochemical reactions often requires the presence of essential metal ions but the biological functions of nucleotides are inhibited by the presence of toxic metals ions. When studying biological and environmental processes where these substances are involved, their

binding capacity towards metal ions cannot be neglected. As is well known, the structure of every nucleotide is constituted by a nucleoside, formed by a purine or pyrimidine base bound to a ribose molecule, which is bound to a mono- di- or tri- phosphate, usually at the 5 position of ribose, and for different nucleotides different complexes can be formed.

The solution chemistry of nucleotides mainly depends on the binding capacity of phosphate groups, in particular the terminal one; in some cases, depending on pH conditions, the nitrogen of the base, generally N-7, participates in metal complexation.

Many papers have been published on metal-nucleotide complexation, especially regarding the interaction of ATP with alkaline earth metal ions (Ca^{2+} and Mg^{2+}), and a number of compilations (Martell and Smith, 1981; Murray and May, 2000; Perrin, 1979; Siegel and Siegel, 1996; Smith and Martell, 1976;

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Smith *et al.*, 1991, 1997) are available where recommended and tentative protonation and formation constant values for the M(AMP/ADP/ATP) systems are reported at different ionic strengths (mostly 0.1 mol L⁻¹) and in different ionic media. In spite of such large volumes of results, no data are reported for mixed electrolyte solutions and therefore literature data are not useful in speciation studies of nucleotides in natural waters where different cations are present simultaneously and in different concentrations depending on the type of aquatic ecosystems (fresh waters, saline waters or seawaters) being studied.

With the aim of furthering our understanding of the chemical speciation of organic matter in natural waters, we report the results we obtained for the speciation of some nucleotide mono-, di- and triphosphates in a multi-component ionic medium (Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, SO₄²⁻) simulating the macro-composition of seawater (Synthetic SeaWater for Equilibrium studies (SSWE), De Robertis *et al.*, 1994; De Stefano *et al.*, 1994). The systems investigated were: adenosine and Inosine 5'-monophosphates (AMP and IMP, respectively); Adenosine 5'-diphosphate (ADP); adenosine 5'-triphosphate (ATP). The protonation constants of nucleotides were determined at *S* (salinity) = 5, 15, 25, 35 and 45‰ using a chemical speciation model that takes all interactions into account and the results were compared to the protonation parameters calculated from single electrolyte systems. A simpler model treating SSWE as a single salt (De Stefano *et al.*, 1998) was also used to calculate the overall complexing ability of the sea water salt for all the systems investigated. As expected, there is a close relationship between the complexing ability of sea salt and the stability of Mg²⁺ and Ca²⁺ complexes.

METHODOLOGY

Materials

Nucleotide 5' mono-, di- and triphosphates (AMP, IMP, ADP, and ATP), all in the form of disodium salt, were supplied by Acros Organic. Their purity, ranging from 97 to 99%, was checked potentiometrically. The purity value of each nucleotide was taken into account in the calculations carried out to determine protonation and complex species formation. Hydrochloric acid and sodium hydroxide solutions were standardised against sodium carbonate and potassium biphthalate, respectively. Tetraethylammonium iodide was re-crystallised from methanol-acetone and the solvent was completely eliminated before use. Tetramethylammonium hydroxide solutions, used as titrants when the background salt was tetraethylammonium iodide, were used as purchased without further purification (Fluka, purissimum). The sodium, calcium, potassium and magnesium chlorides and sodium sulphate used to prepare synthetic seawater

solutions were always dried before use. Calcium and magnesium chlorides were standardised against EDTA. All the solutions were prepared using CO₂-free freshly prepared distilled water ($R = 18 \Omega \text{ cm}^{-1}$).

Experimental equipment and procedure

The measurements were carried out using potentiometric apparatus consisting of a Metrohm mod. 665 automatic titrant dispenser coupled with a Metrohm mod. 654 potentiometer and a combination Orion-Ross 8172 glass-electrode. The estimated accuracy of this system was $\pm 0.15 \text{ mV}$ and $\pm 0.003 \text{ mL}$ for e.m.f. and titrant volume readings, respectively. Pure nitrogen was bubbled through the solutions in the titration cells in order to avoid O₂ and CO₂ inside and the solutions were magnetically stirred. In order to adjust the ionic strength to different values, a volume of 20–25 ml of solution containing the nucleotide (2–5 mmol L⁻¹) under investigation and the background salt (Et₄NI, NaCl, NaCl/CaCl₂, NaCl/MgCl₂ and Synthetic Seawater) was titrated with hydroxide solutions (NaOH, Me₄NOH). For each experiment, independent titrations of hydrochloric acid with NaOH or Me₄NOH solutions were performed in the same experimental conditions of ionic strength as the systems under study in order to determine formal electrode potential. All titrations were carried out up to pH \approx 11.

Calculations

The non linear least squares computer program ESAB2M (De Stefano *et al.*, 1987) was used to determine all the parameters of an acid – base titration (analytical concentration of the reagents, electrode potential, junction potential coefficient j_a , ionic product of water K_w). The following additional computer programs (De Stefano *et al.*, 1997) were also used: BSTAC and STACO to calculate protonation and formation constants, and ES4ECI to draw distribution diagrams of the species in solution and to compute species formation.

RESULTS AND DISCUSSION

Protonation of Nucleotides in different ionic media.

To evaluate the influence of sodium, which is by far the most important component of seawater, on the protonation constants of the nucleotides under investigation, the apparent protonation constants of AMP and ADP were determined in NaCl and, for comparison, in tetraethylammonium iodide ionic media and in the ionic strength range $0 \leq I(\text{mol L}^{-1}) \leq 1$. Results are reported in Table 1. The results obtained are in very good agreement with literature data. Smith *et al.* (1991) report the following recommended values: $\log K^{\text{H}_1} = 6.18 \pm 0.08$; $\log K^{\text{H}_2} = 3.80 \pm 0.07$ for AMP-5 and $\log K^{\text{H}_1} = 6.31 \pm 0.09$ and $\log K^{\text{H}_2} = 3.94 \pm 0.05$ for ADP-5 system, at

Table 1 Protonation constants of AMP and ADP in Et₄Ni and NaCl ionic media at T = 25°C

I/mol L ⁻¹	Medium	AMP		ADP	
		logK ₁ ^H	logK ₂ ^H	logK ₁ ^H	logK ₂ ^H
0.1	NaCl	6.176 ± 0.004 ^a	3.773 ± 0.005	6.38 ± 0.01 ^a	3.915 ± 0.009
0.25		6.042 ± 0.003	3.756 ± 0.005	6.17 ± 0.01	3.851 ± 0.008
0.50		5.944 ± 0.003	3.789 ± 0.005	6.017 ± 0.009	3.870 ± 0.009
0.75		5.894 ± 0.005	3.846 ± 0.006	5.932 ± 0.008	3.94 ± 0.01
1.0		5.866 ± 0.007	3.914 ± 0.009	5.88 ± 0.01	4.02 ± 0.02
0.1	Et ₄ Ni	6.276 ± 0.004	3.736 ± 0.006	6.52 ± 0.02	3.869 ± 0.009
0.25		6.273 ± 0.004	3.662 ± 0.004	6.48 ± 0.01	3.735 ± 0.007
0.50		6.365 ± 0.006	3.601 ± 0.002	6.57 ± 0.02	3.638 ± 0.007
0.75		6.478 ± 0.009	3.565 ± 0.002	6.69 ± 0.03	3.59 ± 0.01

^aSD

0.1 mol L⁻¹ in NaCl. LogK^H values obtained in Et₄N⁺ ionic medium (0.1 mol L⁻¹) are also consistent with data reported by the same authors [logK^H₁ = 6.34 ± 0.05 (tentative value) for the AMP-5 system and logK^H₁ = 6.63 ± 0.05 (recommended) for ADP-5 system].

Apparent protonation constants for ATP and IMP in the same ionic media, excluding KCl and tetrapropylammonium chloride media, were taken from literature. Some of these values are reported in Table 2.

Protonation constants of nucleotides in synthetic seawater

Investigations were also carried out in a multi-component ionic medium (Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻ and SO₄²⁻) simulating the composition of natural seawater. Table 3 shows the composition of this medium (Synthetic Seawater for Equilibrium studies, SSWE) at different salinity values. Component concentrations are expressed in mol L⁻¹, at T = 25°C.

The protonation constants of nucleotide mono-, di- and triphosphates were determined in SSWE in the salinity range 5–45‰. Results are reported in Table 4.

Table 2 Protonation constants of ATP and IMP in different ionic media at T = 25°C

I/mol L ⁻¹	Medium	ATP ^a		IMP	
		logK ₁ ^H	logK ₂ ^H	logK ₁ ^H	
0.1	KCl ^b	–	–	6.19	
0.16	NaCl	6.31	10.25	–	
0.25		6.16	10.08	–	
0.49		5.96	9.89	–	
0.97		KCl ^c	–	–	5.91
1.0	Et ₄ Ni	5.82	9.76	–	
0.1		Pr ₄ N ⁺ ^b	–	–	6.34
0.16		6.88	10.77	–	
0.25		6.85	10.69	–	
0.49		6.88	10.66	–	
1	7.00	10.67	–		

^aDe Robertis *et al.*, 1986; ^bSmith *et al.*, 1991; ^cMakitie and Mirttinen, 1971.

Table 3 Composition of SSWE at different salinity values

Component	Salinity (‰)				
	5	15	25	35	45
NaCl	0.059	0.1782	0.2992	0.4221	0.5467
Na ₂ SO ₄	0.004	0.0122	0.0204	0.0288	0.0373
KCl	0.0015	0.0046	0.0078	0.0110	0.0142
CaCl ₂	0.0015	0.0047	0.0078	0.0111	0.0143
MgCl ₂	0.0077	0.0231	0.0389	0.0548	0.0710

Table 4 Apparent protonation constants of nucleotides in artificial seawater at different salinity values and at T = 25°C

Salinity (‰)	System	LogK ₁ ^{H a}	logK ₂ ^{H a}
AMP			
5		6.00 ± 0.05	3.81 ± 0.03
15		5.85 ± 0.05	3.80 ± 0.03
25		5.78 ± 0.03	3.79 ± 0.03
35		5.76 ± 0.04	3.85 ± 0.04
45		5.66 ± 0.04	3.92 ± 0.05
IMP			
5		6.03 ± 0.04	–
15		5.85 ± 0.03	–
25		5.78 ± 0.03	–
35		5.73 ± 0.02	–
45		5.67 ± 0.03	–
ADP			
5		5.36 ± 0.04	3.93 ± 0.02
15		5.08 ± 0.03	3.81 ± 0.03
25		4.99 ± 0.03	3.84 ± 0.02
35		4.92 ± 0.04	3.90 ± 0.03
45		4.85 ± 0.04	3.98 ± 0.04
ATP			
5		5.07 ± 0.02	3.83 ± 0.03
15		4.79 ± 0.02	3.63 ± 0.04
25		4.77 ± 0.03	3.63 ± 0.03
35		4.72 ± 0.04	3.63 ± 0.04
45		4.60 ± 0.04	3.66 ± 0.04

^aSD

Dependence of protonation constants on ionic strength

In speciation studies of natural waters a wide range of ionic strengths have to be considered: from $\approx 0.08 \text{ mol L}^{-1}$ (for lakes) up to 0.7 mol L^{-1} (seawater) and more for seawater and hypersaline waters. Literature data for the protonation and complex species formation of the systems we are investigating report only one ionic strength value, *i.e.* mainly 0.1 mol L^{-1} , and are therefore not useful in performing speciation studies. The constant values we determined at different ionic strengths (Tables 1–3 and 5) allow us to define the dependence on ionic strength of the protonation and formation constants of nucleotides in all the ionic media used as background salts, and to calculate protonation and formation constant values at every ionic strength value in the range $0 \leq I(\text{mol L}^{-1}) \leq 1$.

Dependence on ionic strength was taken into account by using the Extended Debye–Hückel type equation (EDH) [the term $A z^* I^{1/2} (1 + 1.5 I^{1/2})^{-1}$ was simplified, by keeping $A = 0.5$, to $z^* I^{1/2} (2 + 3 I^{1/2})^{-1}$] (Daniele *et al.*, 1991, 1997):

$$\log K = \log {}^T K - z^* I^{1/2} (2 + 3 I^{1/2})^{-1} + CI + DI^{3/2} \quad (1)$$

where K is the protonation constant; ${}^T K$ is the protonation constant at infinite dilution; C and D are empirical parameters that can be expressed as:

$$C = c_0 p^* + c_1 z^* \text{ and } D = d_0 p^* + d_1 z^*$$

with

$$p^* = \frac{\sum (\text{moles})_{\text{reactants}} - \sum (\text{moles})_{\text{products}}}{\sum (\text{charges})_{\text{reactants}}^2 - \sum (\text{charges})_{\text{products}}^2}$$

where the empirical parameters c_0 , c_1 , d_0 , d_1 are dependent only on the stoichiometry of the reaction in the range $0 < I (\text{mol L}^{-1}) < 1$ (Daniele *et al.*, 1997):

$$C = 0.1 p^* + 0.20 z^* \text{ and } D = -0.075 z^*$$

For the protonation constants studied in this work we obtained the empirical parameters reported in Table 5, which are in agreement with the above general parameters.

By using eq. (1) with the parameters in Table 5, protonation constants at infinite dilution (${}^T K$) were calculated for all the nucleotides under investigation. Results are reported in Table 6.

The best fit for the dependence of nucleotide protonation constants on salinity is given by

$$\log K_j^H = \log {}^T K_j^H + a S^{1/2} + bS - \log(1 + cS) \quad (2)$$

where: a , b and c are empirical parameters.

Table 5 Parameters for the ionic strength dependence of protonation constants (eq. 1) for AMP and ADP

	AMP		ADP	
	NaCl	Et ₄ NI	NaCl	Et ₄ NI
log K ₁ ^H C	0.06 ± 0.02 ^a	1.15 ± 0.05 ^a	0.067 ± 0.005 ^a	1.62 ± 0.02 ^a
D	0	-0.40	0	-0.60
log K ₂ ^H C	0.33 ± 0.04	0.01 ± 0.02	0.56 ± 0.02	0.07 ± 0.02
D	0	0	0	0

^aSD

Table 6 Protonation constants of nucleotides at $I = 0 \text{ mol L}^{-1}$ and $T = 25^\circ\text{C}$

	log K ₁ ^H ± 3S ^a	log K ₂ ^H ± 3S
ATP ^b	7.65	4.53
ADP	7.03 ± 0.05 (7.01 ± 0.05) ^c	4.30 ± 0.03 (4.28 ± 0.04) ^c
AMP	6.61 ± 0.04 (6.65 ± 0.05) ^c	3.96 ± 0.03 (4.00 ± 0.04) ^c
IMP	6.65 ^c	–

^aSD; ^bDe Robertis *et al.*, 1986; ^ccalculated in this study by taking into account several literature values reported by Smith *et al.*, 1991

Table 7 shows parameters for the dependence on ionic strength of protonation constants in SSWE at $T = 25^\circ\text{C}$.

Figures 1(a)–(c) show the dependence on ionic strength of protonation constants for the AMP, ADP and ATP systems in Et₄NI, NaCl and SSWE ionic media.

Examination of Figures 1(a)–(c) shows that:

1. Protonated species of all the nucleotides under study are most stable in the Et₄NI ionic medium. Therefore, Et₄NI can be considered the baseline non-interacting ionic medium.
2. The lower protonation constants in NaCl than in Et₄NI might be due to the nucleotides forming weak sodium complex species (De Robertis *et al.*, 1986).
3. The considerably lower protonation constants observed in SSWE can be attributed to a further contribution to complexation made by calcium and magnesium

Table 7 Parameters for the dependence on ionic strength of protonation constants in SSWE at $T = 25^\circ\text{C}$

System	j	a	b	c
AMP	1	0.13 ± 0.01 ^a	–	1.2 ± 0.2
	2	-0.097 ± 0.004	0.013 ± 0.001	–
IMP	1	0.12 ± 0.01	–	1.2 ± 0.2
	2	-0.226 ± 0.007	0.027 ± 0.001	–
ADP	1	0.102 ± 0.008	–	15.0 ± 1.3
	2	-0.226 ± 0.007	0.027 ± 0.001	–
ATP	1	0.12 ± 0.01	–	142.2 ± 14.4
	2	-0.379 ± 0.008	0.038 ± 0.002	–

^aSD

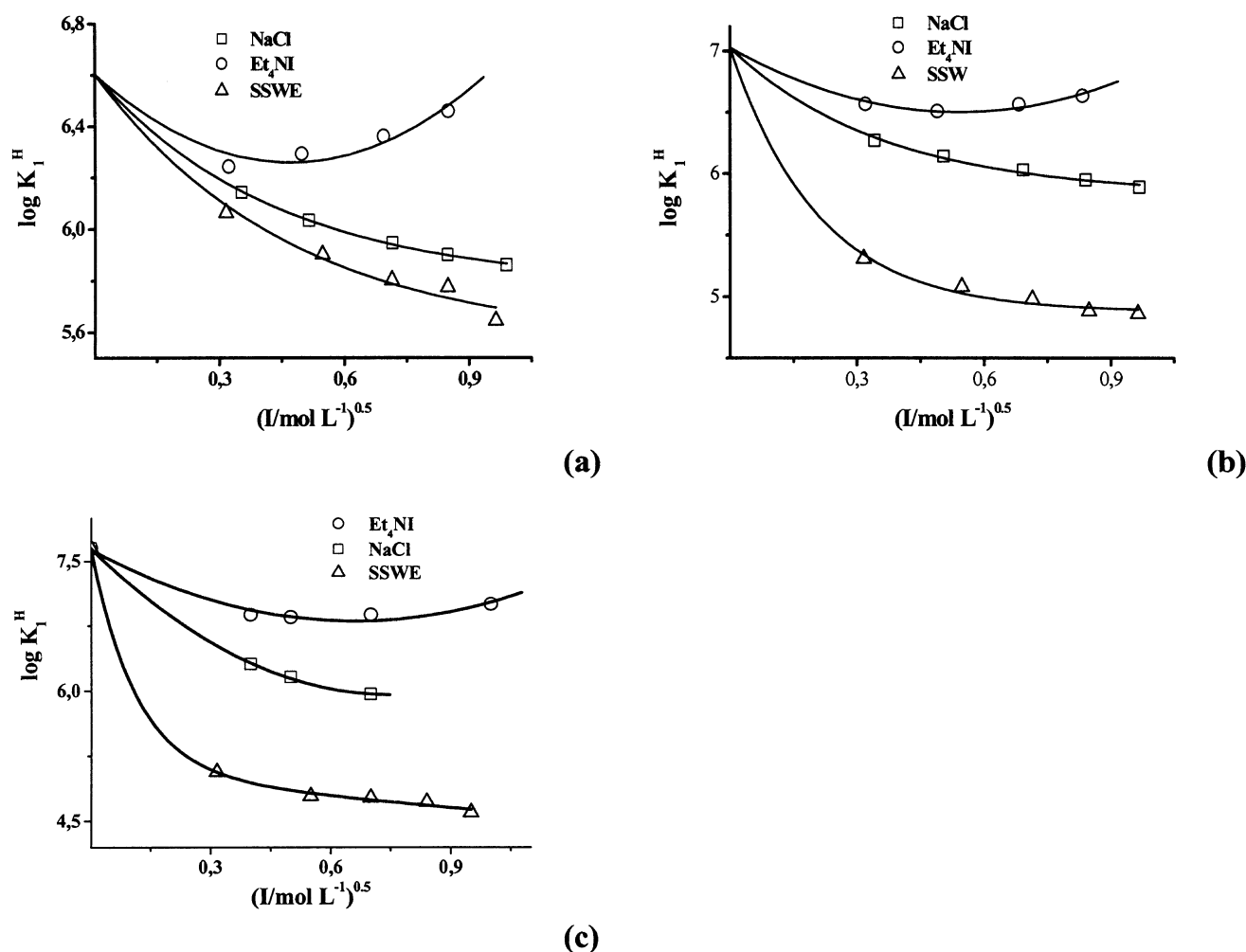


Figure 1 Dependence on ionic strength of $\log K_1^H$ of AMP (a), ADP (b) and ATP (c) in Et_4NI , NaCl and SSWE ionic media.

ions, which form more stable nucleotide complexes than sodium-nucleotides one.

Formation constants of nucleotides with the major cationic components of seawater

After protonation constants were determined in ionic media containing the major cationic components of natural waters, the formation constants of nucleotide complexes with these cations were determined in sodium, calcium and magnesium single salt electrolyte solutions and in a wide range of ionic strengths. Although sodium ions interact weakly with nucleotides, this interaction cannot be neglected when dealing with chemical speciation studies in seawater where sodium concentration is far higher than concentrations of all other components. Tentative formation constant values for weak sodium nucleotide complexes have been reported in literature (De Robertis *et al.*, 1986; Smith *et al.*, 1991). Complexes of nucleotides with calcium and magnesium ions are stronger than sodium ion complexes and have been extensively investigated. Formation constants are reported in a number of papers and com-

pilations, but generally only at a single ionic strength value; these are therefore not useful for the purposes of chemical speciation studies. Here we report the formation constants of calcium and magnesium nucleotide complexes determined in mixed $\text{NaCl}/\text{MgCl}_2$ and $\text{NaCl}/\text{CaCl}_2$ media in the ionic strength range $0.1 \leq I(\text{mol L}^{-1}) \leq 1$. Results are shown in Tables 8 and 9. These formation constants will be compared with the formation constants obtained in the study of nucleotide interactions

Table 8 Formation constants for calcium and magnesium – AMP and -IMP complexes in mixed ionic media^a at different ionic strengths and $T = 25^\circ\text{C}$

$I/\text{mol L}^{-1}$	$\log K_{\text{Ca}(\text{AMP})}$	$\log K_{\text{Mg}(\text{AMP})}$
0	2.28 ± 0.05^b	2.40 ± 0.05
0.1	1.43 ± 0.04	1.56 ± 0.03
0.25	1.16 ± 0.03	1.29 ± 0.02
0.50	0.95 ± 0.03	1.09 ± 0.02
0.75	0.85 ± 0.03	0.98 ± 0.02
1.0	0.79 ± 0.04	0.92 ± 0.03

^a NaCl/XCl_2 ; X = Ca^{2+} or Mg^{2+} ; ^bSD

Table 9 Formation constants for calcium and magnesium – ADP complexes in mixed ionic media^a at different ionic strengths and $T = 25^\circ\text{C}$

$I/\text{mol L}^{-1}$	$\log K_{\text{Ca(ADP)}}$	$\log \beta_{\text{Ca(ADP)H}}$	$\log K_{\text{Mg(ADP)}}$	$\log \beta_{\text{Mg(ADP)H}}$
0	4.19 ± 0.03^b	8.84 ± 0.04	4.54 ± 0.02^b	8.72 ± 0.04
0.1	2.92 ± 0.03	7.43 ± 0.04	3.27 ± 0.02	7.32 ± 0.04
0.25	2.55 ± 0.02	7.11 ± 0.03	2.90 ± 0.01	7.00 ± 0.02
0.50	2.30 ± 0.02	7.02 ± 0.02	2.661 ± 0.007	6.90 ± 0.01
0.75	2.21 ± 0.01	7.09 ± 0.02	2.56 ± 0.01	6.977 ± 0.008
1.0	2.18 ± 0.01	7.23 ± 0.01	2.53 ± 0.02	7.12 ± 0.02

^aNaCl/XCl₂; X = Ca²⁺ or Mg²⁺; ^bSD

with a multicomponent ionic medium simulating the composition of seawater (SSWE) where calcium and magnesium ions are present in different concentrations depending on the salinity value considered.

Single salt seawater complexes

Measurements made in a SSWE ionic medium where sodium, potassium, calcium and magnesium ions are present simultaneously (see Table 3) allow us to define speciation profiles for the nucleotides under investigation in seawater. When a SSWE multi-component ionic medium is used as a background salt in speciation studies, the internal ionic interactions between the components cannot be neglected because, to different extents, they lower the free ion concentration owing to the formation of the following 8 species: NaCl⁰, KCl⁰ (very weak, $\log K < 1 \text{ mol L}^{-1}$), MgCl⁺, CaCl⁺, Na(SO₄)⁻, Mg(OH)⁺ (weak), and Mg(SO₄)⁰ and Ca(SO₄)⁰ (fairly strong). If to these species we add the other protonated and complex species formed in the nucleotide systems, a significant number of species needs to be considered. To simplify equilibrium calculations, we recently proposed a more straightforward approach (De Stefano *et al.*, 1998) designed to take into account all the interactions among the major components of seawater by considering the components of synthetic seawater (SSWE) as a single 1:1 salt (BA) whose concentration is $C_{\text{BA}} = 1/2\sum C_i$ (C_i = concentration of all the ions) with z ion charge calculated as $z = \sqrt{I/C_{\text{BA}}} = \pm 1.117$. The use of the single salt approximation (valid in the range $5 \leq S\% \leq 45$) considerably reduces the complexity of the systems to be investigated. Indeed, only three species deriving from internal ionic medium interactions need be considered: BA⁰ from self association, HA^(1-z) from anion protonation and B(OH)^(z-1) from cation hydrolysis. The formation constants of these species, together with the composition of the single salt BA at different salinities are reported in Table 10.

For the above reasons, the speciation of nucleotides in SSWE was studied using the single salt (BA) approximation and by including the above equilibrium constants for the BA system (Table 10) in the calculations carried out to quantitatively determine the interactions of nucleotide species with the cation B^{z+} and the anion A^{z-},

which are respectively representative of all the major cations (Na⁺, K⁺, Ca²⁺ and Mg²⁺) and anions (Cl⁻ and SO₄²⁻) in seawater salt. Calculations performed using STACO and BSTAC computer programs provided evidence of formation of the species reported in Table 11.

The formation constant values obtained are lower than those determined in a single electrolyte medium for calcium and magnesium nucleotide complexes (Table 9) and much higher than those of sodium complexes. For example, at $I = 0.5 \text{ mol L}^{-1}$ and $T = 25^\circ\text{C}$, we have $\log K(\text{NaATP}) = 1.36$ (De Robertis *et al.*, 1986); other stability values for alkali metal nucleotide complexes are reported in the Critical Evaluation of Smith *et al.* (1991). This is consistent with the meaning of the single *seasalt* model according to which

Table 10 SSWE as a “single salt”. Composition at different salinity values and equilibrium constants.

Component	Salinity (S‰)				
	5	15	25	35	45
BA ^a	0.0803	0.2428	0.4078	0.5751	0.7449
$I (\text{mol L}^{-1})$	0.100	0.303	0.509	0.717	0.929
Equilibrium	$\log K^b$				
B ^{z+} + A ^{z-} = BA ⁰	-0.03				
H ⁺ + A ^{z-} = HA ^(1-z)	0.24 ± 0.05				
B ^{z+} = B(OH) ^(z-1) + H ⁺	-12.75				

^aSingle salt; ^b at $I = 0 \text{ mol L}^{-1}$ and $T = 25^\circ\text{C}$

Table 11 Formation constants of nucleotide 5' mono-, di- and triphosphates with “seasalt” BA, at 35‰ salinity and at infinite dilution ($I = 0 \text{ mol L}^{-1}$) and $T = 25^\circ\text{C}$.

System	Equilibrium ^a	$\log \beta$ ($S = 35\%$)	$\log \beta$ ($I = 0$)
AMP	B + AMP = B(AMP)	0.85 ± 0.04^b	1.20 ± 0.04
IMP	B + IMP = B(IMP)	0.89 ± 0.04	1.43 ± 0.05
ADP	B + ADP = B(ADP)	2.02 ± 0.03	2.61 ± 0.04
ATP	B + ATP = B(ATP)	3.35 ± 0.03	3.88 ± 0.04
	A + ATP + H = A(ATP)H	7.5 ± 0.1	7.6 ± 0.2
	2B + ATP = B ₂ (ATP)	2.8 ± 0.1	3.9 ± 0.2
	B + ATP + H = B(ATP)H	7.90 ± 0.05	8.59 ± 0.05

^aCharges omitted for simplicity; ^b ± 3 S.D.

cation B broadly represents the chemical behaviour of sodium ions and to a lesser extent the behaviour of calcium and magnesium ions.

Speciation profiles of nucleotides in seawater

Using the data reported in Table 11 and the protonation constants of nucleotides in artificial seawater (Table 4), speciation diagrams for all the systems investigated in ‘single salt’ SSWE were generated by the ES4ECI computer program. Figures 2–5 show species distribution for AMP, IMP, ADP and ATP both in SSWE (as a single salt, BA) at 35‰ salinity where, in addition to the protonated species, B- and A-nucleotides species are also considered, and at $I = 0 \text{ mol L}^{-1}$, i.e. in the absence of any interaction with the BA salt.

In all systems investigated the percentage formation of BL (L = AMP, IMP, ADP or ATP) species is very high

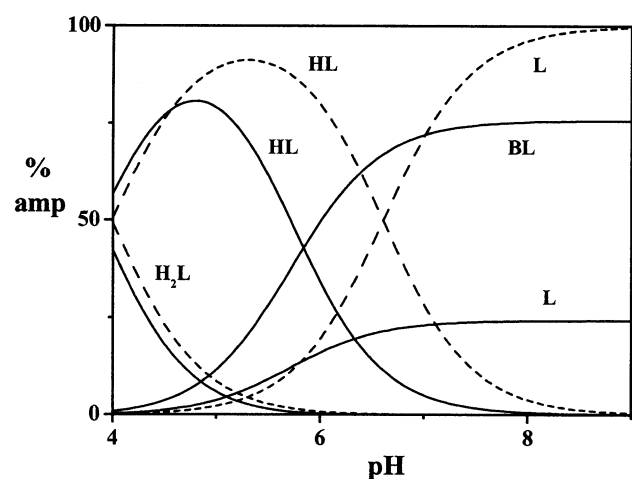


Figure 2 Speciation diagram of AMP (L) in SSWE as a single seasalt (BA) at 35‰ salinity (continuous line) and at $I = 0 \text{ mol L}^{-1}$ (dotted line). $C_L = 1 \text{ mmol L}^{-1}$.

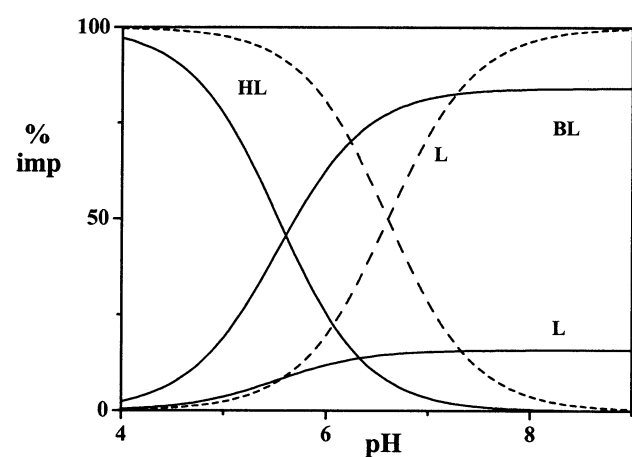


Figure 3 Speciation diagram of IMP (L) in SSWE as a single seasalt (BA) at 35‰ salinity (continuous line) and at $I = 0 \text{ mol L}^{-1}$ (dotted line). $C_L = 1 \text{ mmol L}^{-1}$.

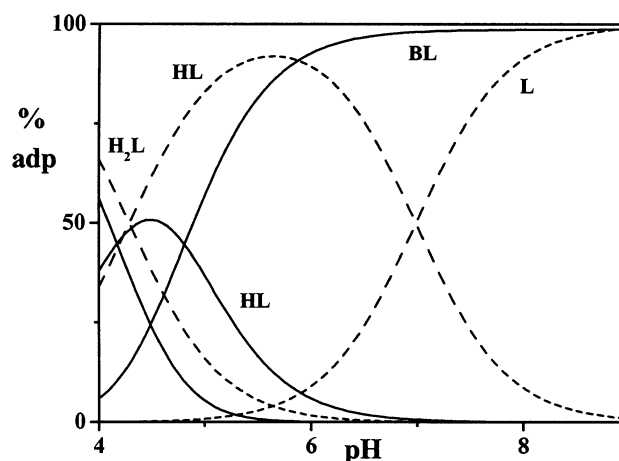


Figure 4 Speciation diagram of ADP (L) in SSWE as a single seasalt (BA) at 35‰ salinity (continuous line) and at $I = 0 \text{ mol L}^{-1}$ (dotted line). $C_L = 1 \text{ mmol L}^{-1}$.

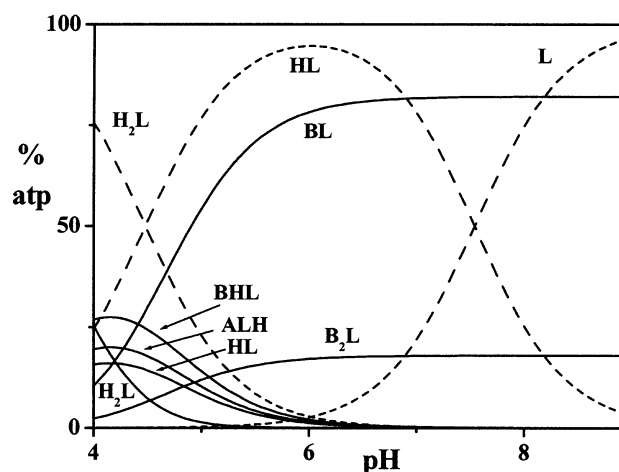


Figure 5 Speciation diagram of ATP (L) in SSWE as a single seasalt (BA) at 35‰ salinity (continuous line) and at $I = 0 \text{ mol L}^{-1}$ (dotted line). $C_L = 1 \text{ mmol L}^{-1}$.

in the pH range (6.5–8.5) of interest for natural waters. At the pH value of seawater (8.1 ± 0.2) BL species formation is over 70% for AMP, IMP and ATP systems, and 100% for the ADP system. It is important to note that for the ATP–BA system, in addition to BL, the B_2L species was found with percentage formation of about 18%. The sum of the two species brings ATP–seawater cation complex formation up to 100%. Other minor species, such as H_xL ($x = 1$ or 2) as well as BHL and ALH are formed outside the acidic pH range of interest for seawater

CONCLUSIONS

The main conclusions of this work can be summarised as follows:

- (1) Results obtained relative to the acid–base behaviour of nucleotide mono-, di- and tri-phosphates in a multi-component ionic medium are the first reported in literature;
- (2) Investigations into the ionic strength dependence of the protonation and formation constants of nucleotides allow us to define the chemical speciation of these compounds in all natural waters with ionic strength ranging between 0.1 and 1 mol L⁻¹;
- (3) The use of a simpler model according to which the major ion components of seawater are considered as a single *seasalt*, made it possible to facilitate calculations and to quantitatively evaluate the cumulative inorganic binding capacity of seawater;
- (4) At the pH value of seawater (8.1 ± 0.2), most of the nucleotides under investigation are present as complex species with cation B of the single *seasalt*, and their formation reaches 100% in the ADP and ATP systems.

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