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Thermodynamic interactions of the alkaline earth metal ions with citric acid

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Abstract By using the isothermal titration calorimetry (ITC) technique, thermodynamic parameters have been determined for reactions of the Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} ions with the citrate anion. The measurements were run in the Cacodylate, Pipes and Mes buffer solutions of a pH of 6, at 298.15 K, as well as in the Tricine, Tapso, and Tris–HCl buffer solutions of a pH of 8. Further, based on the results of potentiometric titration, the conditional stability constants were determined for the citrate complexes at both pH values. The effect of the reaction environment and the metal ion identity on the interaction energy with the citrate ligand and the stability of the resulting compounds have been discussed.

Keywords Citrate complexes · Thermodynamic parameters · Isothermal titration calorimetry

Introduction

Both citric acid (H_3Cit) and its salts occur in small quantities in the majority of living organisms to play an important role in biological processes [1–3]. Citric acid is a crucial intermediate in the Krebs cycle and is an essential compound in a number of metalloenzyme active sites. Owing to its complex-forming capacity and lack of toxicity, citrates have extensively been used, amongst others, in the food and pharmaceutical industries as well as in medicine as chelating agents, and are implemented in various biochemical processes. The presence of a hydroxyl group and three carboxyl functions in the structure of the compound enables the formation of thermodynamically stable complexes with a variety of metal ions, e.g., Mg^{2+}, Ca^{2+}, Sr^{2+}, Mn^{2+}, Co^{2+}, and La^{3+} [4–10]. Binding of the metal ions to citric acid enhances their bioavailability and facilitates successive absorption of the ions by biological systems [11].

In acidic and neutral solutions it dissociates in three steps represented by the equations (1–3):

$$H_3Cit + H_2O = H_2Cit^- + H_3O^+ \qquad K_{a1} = \frac{[H_2Cit^-][H_3O^+]}{[H_3Cit]} \tag{1}$$

$$H_2Cit^- + H_2O = HCit^{2-} + H_3O^+ \qquad K_{a2} = \frac{[HCit^{2-}][H_3O^+]}{[H_2Cit^-]} \tag{2}$$

$$HCit^{2-} + H_2O = Cit^{3-} + H_3O^+ \qquad K_{a3} = \frac{[Cit^{3-}][H_3O^+]}{[HCit^{2-}]}$$

Equilibrium concentrations of particular forms resulting from these equations determine the dissociation constants of the acid. At 298.15 K respective equilibrium constants pK_{a1}, pK_{a2}, and pK_{a3} are 3.13, 4.76, and 6.4 [12, 13].

Solid phase studies [14–17] have shown that a proton of the central carboxyl group (α to a hydroxyl group) is split first followed by protons of the terminal carboxyls (Fig. 1). Inspection of the NMR spectra suggests that a similar dissociation pattern occurs in solution [18].

In strongly basic solutions, also a hydroxyl proton is split with a pK_{a4} equal 11.6 [13]. The coordination of metal ions to the citrate ion significantly decreases the pK_{a4} value of the hydroxy group, thus enhancing the metal ion–ligand

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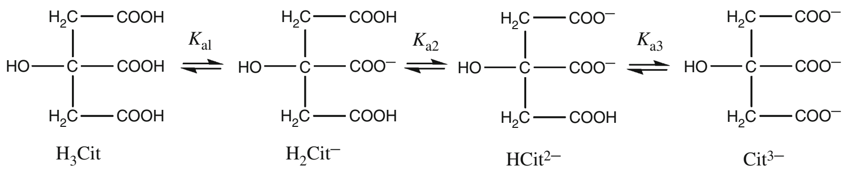

Fig. 1 Dissociation equilibria of citric acid

interaction [19, 20]. In the majority of cases of complex formation, ionization of the citrate hydroxyl can be neglected, since due to the large pK_{a4} value it does not undergo deprotonation under physiological pH oscillating around 7. For this reason, citrates act as tridentate ligands. The oxygen atoms represented in Fig. 1 act as electron donors. X-ray crystallographic patterns have shown the carboxyl group at the central carbon atom to be almost perpendicular to the carbon backbone and to lie on one plane with the hydroxyl group. Such an arrangement results in a 5-member chelate ring binding a metal ion in a bidentate fashion. A third donor is an oxygen atom of the terminal carboxyl group [21, 22]. A similar coordination pattern can also be expected in solution.

The formation constant of a complex, *K*, contains general information about thermodynamic stability of a species formed. It encompasses all the effects accompanying ligand binding to a substrate. Each reaction is accompanied by a variety of processes, such as conformational changes of the reactants, breaking and formation of hydrogen bonds, electrostatic and hydrophobic interactions and fluctuations in the reaction environment**.** Just for this reason the knowledge of thermodynamic parameters (ΔH, ΔS) of a reaction enables a better understanding of the nature of chemical processes and factors affecting the stability of the resulting compounds than does that of simple equilibrium constant, *K*.

In this contribution, thermodynamic parameters are represented for complexation reactions of the Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} ions with citric acid as determined by the isothermal titration calorimetry (ITC) technique, as well as the formation constants of the complexes determined by potentiometric titration. Also the effect of the reaction environment (pH) and identity of metal ion on the stability of the resulting complexes has been discussed.

Experimental

Materials

All reagents, namely sodium citrate dihydrate, $C_6H_5O_7Na_3 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, $SrCl_2$, $BaCl_2 \cdot 2H_2O$, Cacodylate, Pipes, Mes, Tricine, Tapso, and Tris were purchased from Aldrich Chemical Corp. and used as received.

Isothermal titration calorimetry

All ITC experiments were performed at 298.15 K using an AutoITC isothermal titration calorimeter (MicroCal Inc., Northampton, USA) with a 1.4491-mL sample and the reference cells. The reference cell contained distilled water. The data, specifically the heat normalized per mole of injectant, were processed with Origin 7 from MicroCal. An initial 2 μL injection was discarded from each dataset in order to remove the effect of titrant diffusion across the syringe tip during the equilibration process. The experiment consisted of injecting 10.02 μL (29 injections, 2 μL for the first injection only) of ca. 10–15 mM solution of appropriate salt into the reaction cell initially containing ca. 1 mM buffered solution of sodium citrate. A background titration, consisting of the identical titrant solution but the buffer solution in the sample cell only, was subtracted from each experimental titration to account for the heat of dilution. All the solutions were degassed prior to titration. The titrant was injected in 5-min intervals to ensure that the titration peak returned to the baseline before the next injection. Each injection lasted 20 s. To ensure homogeneity of mixing in the cell, the stirrer speed was kept constant at 300 rpm.

Potentiometric measurements

Potentiometric titrations were performed using an automatic titratory Cerko Lab® System with a 0.5-mL Hamilton's syringe interfaced with an IBM computer. The titrations were performed automatically by means of a suitable program Cerko Lab System ver. 3.OS-Expert. A constant-speed magnetic stirring was applied throughout. The temperature of the titration cell was kept at 298.15 K by means of a Lauda E100 circulation thermostat. The pH combined electrode was bought from the Schott firm. The electrode was calibrated according to IUPAC recommendation [23]. The solutions of the citrate and the metal cations were prepared directly before measurements. Aliquots (1.5 mL) of sample solution, containing appropriate amounts of the Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} ions and the ligand, were potentiometrically titrated with a standardized NaOH solution either over the pH range 3.2–11.4 or until the onset of precipitate formation. The metal ion concentration ranged from 1×10^{-3} to 1.5×10^{-3} M, and the metal-to-ligand ratios were up to 1:1. A minimum of three pairs of titrations were performed for each metal/ligand system. The stability constants of the complexes were determined using the software CVEQUID [24], by minimization of the differences between the theoretical model and the experimental data, following the method of Gauss–Newton–Marquart for nonlinear equations.

Results and discussion

In general, the stability constant (conditional stability constant) of considered reactions can be defined as:

$$\left[\mathrm{Me(H_2O)_6}\right]^{2+} + w\mathrm{H_3Cit} + x\mathrm{H_2Cit^-} + y\mathrm{HCit^{2-}} + z\mathrm{Cit^{3-}} = \left[\mathrm{Me(H_2O)_3Cit}\right]^- + 3\mathrm{H_2O} + (\Delta n)\mathrm{H^+} \qquad (4)$$

$$K = \frac{[\mathrm{MeCit^-}][\mathrm{H^+}]^{\Delta n}}{[\mathrm{Me^{2+}}][\mathrm{H_3Cit}]^w[\mathrm{H_2Cit^-}]^x[\mathrm{HCit^{2-}}]^y[\mathrm{Cit^{3-}}]^z}$$

where Me denotes Mg^{2+}, Ca^{2+}, Sr^{2+}, or Ba^{2+} ion, Coefficients $w + x + y + z = 1$ are pH-dependent values [25]. Water molecules are omitted for clarity.

The isothermal titration calorimetric data enabled to determine the stoichiometry of the complexes n (n = [metal ion]/[ligand]), the formation constants, K, and the thermodynamic parameters of the reactions. Tables 1 and 2 list the thermodynamic characteristics of interactions of the citrate ion with the Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} ions in buffer solutions of different ionization enthalpies, at pH of 6 and 8, at 298.15 K.

The stoichiometry of the compounds indicates nearly equimolar (1:1) metal/ligand ratios of the reactants. The extent of protonation of the citrate carboxyl groups depends on the solution pH and affects the thermodynamic stability of the complexes. The stability at a pH of 8 is greater than that at a pH of 6. These findings have also been supported by the formation constants of the complexes determined potentiometrically. Table 3 lists true (K) and apparent (K') binding constants. The relationship between K and K' is given by the equation (5)

$$K = K' \cdot \alpha, \qquad (5)$$

where α is the side-reaction coefficient. For the citrate ion α was calculated from the expression (6):

$$\alpha_{\mathrm{cit}} = 1 + [\mathrm{H_3O^+}]/K_{\mathrm{a3}} + [\mathrm{H_3O^+}]^2/K_{\mathrm{a3}}K_{\mathrm{a2}} + [\mathrm{H_3O^+}]^3/K_{\mathrm{a3}}K_{\mathrm{a2}}K_{\mathrm{a1}}, \qquad (6)$$

where K_{a1}, K_{a2}, and K_{a3} are successive dissociation constants of the citric acid.

The apparent binding constants, K', account for the protonation extent of a ligand including concentrations of its all equilibrium forms at a given pH and apparent stability constants (K') calculated for solutions of pH 6 and 8.

At a pH of 6, the observed binding enthalpy, ΔH_{obs}, of the ions to the citrate depends on buffer type and decreases with increasing ionization enthalpy of the buffer (Fig. 2). This is so because ΔH_{obs} value is the sum of all energetic effects accompanying a reaction, i.e., enthalpy due to the heat of complex formation, ΔH_{bind}, which is independent

Table 1 Thermodynamic parameters of metal binding to Na_3Cit in buffer solutions of a pH of 6, at 298.15 K

	Metal ion	K/M^{-1}	ΔG^a/kcal mol^{-1}	ΔH/kcal mol^{-1}	$T\Delta S$/kcal mol^{-1}	Stoichiometry
Cacodylate	Mg^{2+}	$(3.91 \pm 0.02) \times 10^3$	−4.88	4.00 (±0.02)	8.88	0.94 (±0.01)
Pipes	Mg^{2+}	$(3.30 \pm 0.03) \times 10^3$	−4.83	2.66 (±0.02)	7.48	0.86 (±0.01)
Mes	Mg^{2+}	$(5.96 \pm 0.1) \times 10^3$	−5.16	1.89 (±0.01)	7.04	0.90 (±0.01)
Cacodylate	Ca^{2+}	$(4.23 \pm 0.09) \times 10^3$	−4.93	0.47 (±0.01)	5.40	0.90 (±0.01)
Pipes	Ca^{2+}	$(3.33 \pm 0.06) \times 10^3$	−4.80	−0.90 (±0.01)	3.91	0.95 (±0.01)
Mes	Ca^{2+}	$(8.0 \pm 0.2) \times 10^3$	−5.33	−1.42 (±0.01)	3.91	0.93 (±0.01)
Cacodylate	Sr^{2+}	$(1.77 \pm 0.09) \times 10^3$	−4.43	0.31 (±0.02)	4.74	1.01 (±0.03)
Pipes	Sr^{2+}	$(1.18 \pm 0.03) \times 10^3$	−4.18	−1.03 (±0.04)	3.16	0.97 (±0.03)
Mes	Sr^{2+}	$(2.73 \pm 0.06) \times 10^3$	−4.68	−1.41 (±0.01)	3.28	1.03 (±0.01)
Cacodylate	[b]					
Pipes	Ba^{2+}	$(0.82 \pm 0.02) \times 10^3$	−3.97	−1.32 (±0.06)	2.65	0.96 (±0.05)
Mes	Ba^{2+}	$(1.74 \pm 0.03) \times 10^3$	−4.42	−1.69 (±0.01)	2.74	1.00 (±0.01)

[a] The equilibrium binding constant, binding enthalpy and reaction stoichiometry were obtained from ITC experiment by fitting binding isotherms, using nonlinear least-squares procedures, to a model that assumes a single set of identical binding sites. From these experimentally determined parameters, the observed free energy of binding (ΔG_{obs}) and entropy change (ΔS) can be determined using the standard thermodynamic relationship: $\Delta G_{\mathrm{overall}} = \Delta G_{\mathrm{obs}} = -RT\ln K_{\mathrm{obs}} = \Delta_{\mathrm{obs}}H - T\Delta S$, where $\Delta G_{\mathrm{overall}}$ denotes the change in free energy for the overall equilibrium of each ITC titration

[b] Energetic effects due to dilution of the Ba^{2+} solution with the Cacodylate buffer are comparable with those of the metal–ligand interaction. For this reason it was impossible to determine the thermodynamic parameters of the reaction

Table 2 Thermodynamic parameters of metal binding to Na_3Cit in buffer solutions of a pH of 8, at 298.15 K

Buffer	Metal ion[a]	K/M^{-1}	ΔG/kcal mol^{-1}	ΔH/kcal mol^{-1}	$T\Delta S$/kcal mol^{-1}	Stoichiometry
Tricine	Mg^{2+}	$(12.3 \pm 0.1) \times 10^3$	−5.58	3.70 (±0.01)	9.27	0.98 (±0.01)
Tapso	Mg^{2+}	$(10.1 \pm 0.1) \times 10^3$	−5.46	3.91 (±0.01)	9.36	0.95 (±0.02)
Tris–HCl	Mg^{2+}	$(11.4 \pm 0.1) \times 10^3$	−5.53	3.80 (±0.01)	9.33	1.00 (±0.01)
Tricine	Ca^{2+}	$(7.6 \pm 0.5) \times 10^3$	−5.31	1.56 (±0.04)	6.86	1.04 (±0.02)
Tapso	Ca^{2+}	$(11.9 \pm 0.8) \times 10^3$	−5.57	1.18 (±0.01)	6.74	0.88 (±0.01)
Tris–HCl	Ca^{2+}	$(9.8 \pm 0.1) \times 10^3$	−5.45	0.42 (±0.01)	5.87	1.15 (±0.01)
Tricine	Sr^{2+}	$(4.6 \pm 0.1) \times 10^3$	−5.01	0.51 (±0.01)	5.52	0.96 (±0.01)
Tapso	Sr^{2+}	$(4.9 \pm 0.2) \times 10^3$	−5.03	0.43 (±0.01)	5.46	0.96 (±0.01)
Tris–HCl	Sr^{2+}	$(3.8 \pm 0.1) \times 10^3$	−4.87	0.29 (±0.01)	5.16	0.94 (±0.01)
Tris–HCl	Ba^{2+}	$(3.1 \pm 0.5) \times 10^3$	−4.74	−0.06 (±0.01)	4.68	1.35 (±0.05)

[a] Energetic effects of dilution of the Ba^{2+} solution with the Tricine and Tapso buffers are comparable with those of the metal–ligand interaction. For this reason it was impossible to determine the thermodynamic parameters of the reactions

Table 3 Formation constants of the Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} citrate complexes (K) determined by potentiometric titration and apparent stability constants (K') calculated for solutions of pH 6 and 8

Metal ion	K	K' (pH = 6)	K' (pH = 8)
Mg^{2+}	$(13.5 \pm 2.3) \times 10^3$	3.70×10^3	13.17×10^3
Ca^{2+}	$(9.4 \pm 0.2) \times 10^3$	2.57×10^3	9.14×10^3
Sr^{2+}	$(3.8 \pm 0.7) \times 10^3$	1.04×10^3	3.70×10^3
Ba^{2+}	$(3.9 \pm 0.4) \times 10^3$	1.04×10^3	3.75×10^3

of the buffer type, and the enthalpy of proton transfer from the ligand to the buffer [26–28] (Eq. 7):

$$\Delta_{obs}H = \Delta_{bind}H + (\Delta n)\Delta_{ion}H_{buf} \tag{7}$$

where $\Delta_{ion}H_{buf}$ is the enthalpy of buffer ionization and Δn is the number of protons exchanged during binding.

A proton released in the reaction (4) is bound to the buffer: buffer + $\Delta n \cdot H^+ \rightleftarrows$ buffer$\cdot\Delta n\cdot H^+$. A plot of $\Delta_{obs}H$ against $\Delta_{ion}H_{buf}$ is a straight line for a given pH value, whose slope indicates the number of protons exchanged during the reaction. The ionization enthalpies of the buffer used herein are −0.71, 2.68, and 3.54 kcal mol^{-1} for Cacodylate, Pipes, and Mes, respectively [29]. The numbers of protons exchanged during the formation of the citrate complexes at a pH of 6 are 0.47(±0.08), 0.43(±0.04), 0.4(±0.01), and 0.42 for Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}, respectively. For this reason, endothermic effects due to Me^{2+}–Na_3Cit interaction in buffer solutions of positive ionization enthalpies (Mes, Pipes) are reduced by the energy released during proton binding to a buffer component ($\Delta H < 0$) (Fig. 2). With the Cacodylate buffer of negative ionization enthalpy (−0.71 kcal mol^{-1}), the proton transfer during complexation of the Me^{2+} ions results in an increase in ΔH_{obs}.

The enthalpy changes independent of the buffer type, ΔH_{bind}, at a pH of 6 are 3.71(±0.22), 0.18(±0.09), 0.03(±0.02), and −0.18 kcal mol^{-1} for Mg^{2+}/Na_3Cit, Ca^{2+}/Na_3Cit, Sr^{2+}/Na_3Cit, and Ba^{2+}/Na_3Cit, respectively. The similar trend of decreasing of the observed enthalpy with increasing of the enthalpy of buffer ionization is seen at a pH of 8. However, in this case, the energetic contribution to the enthalpic term, due to proton exchange during complex formation is not as significant as at a pH of 6, probably due to a more ionized of three carboxyl groups.

The observed enthalpy of the reaction, $\Delta_{obs}H$, declines with increasing metal atomic mass. This tendency can be seen within the entire set of the complexes studied, independent of the buffer type and the pH. This can be explained in terms of decreasing hydration energy of the metal ions with an increase in their ionic radii [30]. The overall energetic effect of the reaction is determined by a difference between hydration energy of the metal ion and that of its complexation by the citrate. The formation of a metal–ligand bond results in the release of the water molecules from the coordination sphere of the metal ion. This is an endothermic process ($\Delta_{dehyd}H > 0$) whose magnitude is the greater the higher is the hydration energy of the metal ion. During the coordination of a metal ion by a ligand, the energy is released as heat ($\Delta_{bind}H < 0$). In the solution of a pH of 8, the energetic contribution to $\Delta_{obs}H$ due to proton exchange during complex formation is in most cases smaller than in the solution of a pH of 6. The $\Delta_{obs}H$ values are positive and decrease with decreasing ionization potential of the metals. This means that within the set of the complexes studied, the energetic contribution due to metal–ligand interaction, $\Delta_{bind}H$, to the observed enthalpy of the reaction, $\Delta_{obs}H$, is overcompensated by an energetic factor due to dehydration of the ion ($\Delta_{dehyd}H > \Delta_{bind}H$). In the solution of a pH of 6, additional contribution to $\Delta_{obs}H$ is made by an exo-energetic effect due to buffer protonation, $(\Delta n)\Delta_{ion}H_{buf} < 0$. The contribution is the higher; the higher is the enthalpy of buffer ionization

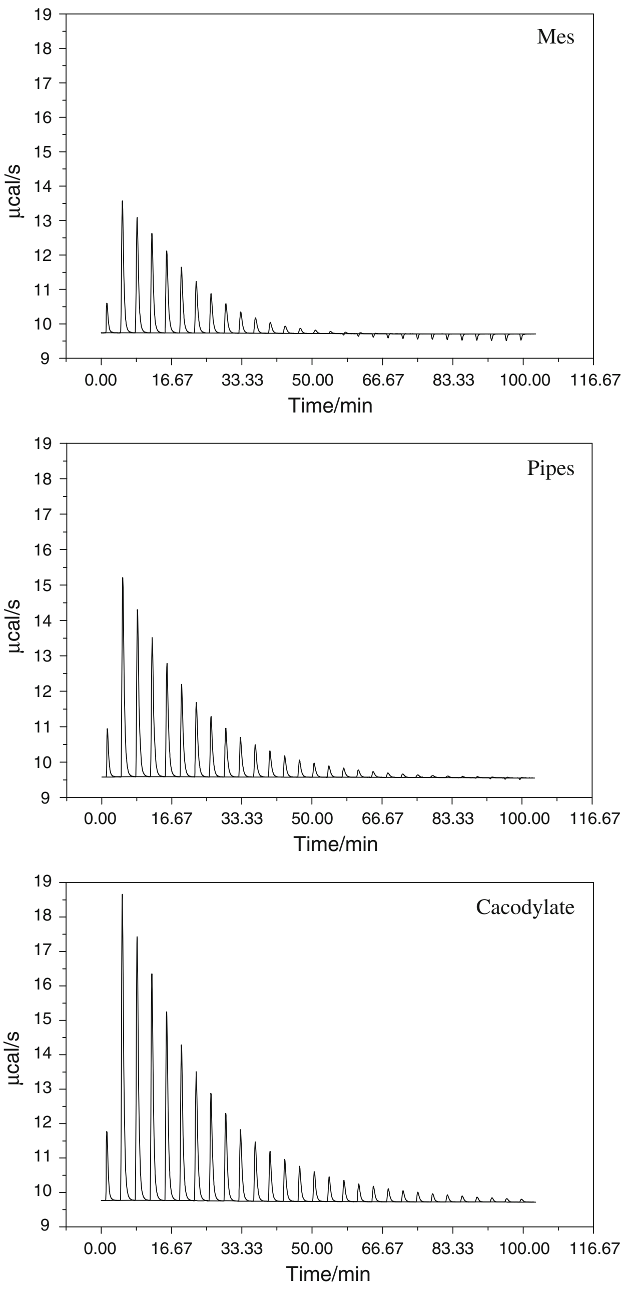

Fig. 2 Mg^{2+}/Na_3Cit titration isotherm in buffers with different ionization enthalpies (20 mM Mes, 20 mM Pipes, 20 mM Cacodylate) at 298.15 K, pH 6.0

and the number of protons exchanged during complex formation.

Of particular importance in the case of chelate-type complexes is the entropy term. In the set of the compounds studied, entropy change increases with increasing hydration capacity of the central ion irrespective of the solution pH (6 or 8). In this case, the increase can be attributed to an increase in solvent entropy due to dehydration of the metal ions during chelate formation. The apparent dynamic hydration number of an ion (that can be defined as the number of tightly bound water molecules that move with an ion as it diffuses, or simply as the number of tightly bound water molecules that must be assigned to an ion to explain its apparent molecular weight) decreases in the order $Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+}$ [31]. The entropy change of the reaction, ΔS, declines also in that direction. In the solution of a pH of 8, the ΔG_{obs} strongly depends on the entropy change of the reaction whose magnitude is larger than that accompanying the formation of analogous compounds in the pH 6 solution. A simple explanation can be offered for this phenomenon. Namely most probably, in the pH 6 solution, owing to a lesser ionization degree of the carboxyls that at a pH of 8, the citrate ligand is more tightly hydrated (ion–dipole interaction) that in the pH 6 solution. This results in a better ordering of the reaction environment in the solvation sphere of the ligand. In addition, conformational (rotational) freedom of a fragment of the ligand not involved in the coordination to the metal ion becomes restricted.

Conclusions

The results obtained by using the ITC technique have shown that the Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} ions form almost equimolar (1:1) compounds with the citrate ligand. This stoichiometry has also been confirmed by the results of potentiometric titration. The interaction energy of the metal ions with the citrate depends on the pH of solution, and the identity of both the buffer and the metal ion. In the solution of a pH of 8, the observed enthalpy of reaction is positive and decreases with increasing ionization potential of a metal. The dehydration energies of the cations also decrease in that direction. An endothermic effect of the reaction of the metal ions with the citrate, in the pH 8 solution, is attributed to a significant input of the dehydration energy of a cation to the observed energy of the reaction, which is overcompensated by an energetic factor due to metal–ligand interaction. In the pH 6 solution, enthalpy of the reaction is negative for the majority of the systems studied. Only with the Mg^{2+} ions, whose dehydration energy is the highest among the ions studied, as well as with the Ca^{2+} and Sr^{2+} ions in the Cacodylate buffer, the enthalpies of the reactions with the citrate are positive. This situation is probably associated with the energy of proton exchange with a buffer component taking place during the reaction with the citrate ion, whose protonation degree is larger at a pH of 6 than at a pH of 8. These assumptions are supported by the observed enthalpies of the reactions which decrease with increasing buffer ionization enthalpy.

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