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Volume and Compressibility Effects in the Formation of Metal-EDTA Complexes

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We used precise measurements of ultrasonic velocity and density to study the complexation of ethylendiaminetetraacetic acid (EDTA) with Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} at 25°C and pH 12. From these measurements we obtained the changes in the molar concentration increment of the ultrasonic velocity ΔA, the apparent molar adiabatic compressibility $\Delta K_{s\Phi}$, and the apparent molar volume ΔV_{Φ} of complex formation. The hydration contributions $\Delta(\Delta V_h)$ to the volume effect of binding range from 39.6 to 46.6 cm^3-mol^{-1} while the hydration contribution to the adiabatic compressibility change in the binding, $\Delta(\Delta K_h)$, ranges from 103.9×10^{-4} to 131.1×10^{-4} cm^3-mol^{-1}-bar^{-1}. These data are interpreted in terms of dehydration of interacting molecules, *i.e.*, transfer of water molecules from the hydration shells of cations and EDTA into the bulk water. The ratio $\Delta(\Delta V_h)/\Delta(\Delta K_h)$ is in the range 0.35 to 0.38 bar, indicating a dominant contribution from the dehydration of charged atomic groups in the volume and the compressibility effects of complex formation.

KEY WORDS: EDTA complexes; alkaline earth metal ions; ultrasonic velocity; compressibility; volume effect; hydration changes.

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1. INTRODUCTION

Formation of complexes of high stability between EDTA and various cations in aqueous solutions is the basis of widespread applications of this multidentate ligand in various fields of science, industry, and medicine.(1) An important application of EDTA in medicine and biology is elimination of radioactive, toxic, harmful, or simply not useful metal cations, both *in vivo* and *in vitro*.(1) While many studies on the stability of EDTA complexes with metal ions have been reported,(2,3) fewer publications on the structure and of the complexes in solution and on the mechanism of complex formation have been published. Little attention has been paid to the thermodynamic properties of the complexes.(4,5) A more detailed knowledge and understanding of complex formation reactions for EDTA are still desirable. In this work we have studied the hydration effects in the complex formation of EDTA and metal ions.

Measurements of volume and compressibility of aqueous solutions are useful techniques to reveal the hydration parameters of solute molecules. We used a combination of high-precision density measurements and resonator ultrasonic velocity measurements to evaluate the volume and compressibility effects in the complex formation of EDTA and metal ions. The high resolution of such measurements has been demonstrated in studies of hydration of nucleic acids and its dependence on the conformation and nucleotide sequence.(6–8) These measurements were used to obtain the structure of metal ion complexes with biopolymers.(6,9–11) In the present work we evaluated the changes of the concentration increment of ultrasonic velocity, apparent molar volume, and apparent molar adiabatic compressibility in the complexation between EDTA and Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} ions. The results were analyzed in terms of hydration characteristics of EDTA and the complexes.

2. EXPERIMENTAL

2.1. Materials

All chemicals were purchased from Merck (Darmstadt, Germany) and were used without further purification. Solutions were prepared using double-distilled water. Solutions were prepared by weight using dry solute samples. The concentrations were further checked by measuring the density and the ultrasonic velocity of the solutions and comparing the results obtained with literature data.(12,13) All experiments were carried out at EDTA concentration of 0.003–0.004*M*. At these concentrations the values of the concentration increment of ultrasonic velocity and apparent molar volume of EDTA can be equated with the values at infinite dilution, within the limits of experimental error. All measurements were done at 25°C and pH 12. Under these conditions carboxylic groups of EDTA are deprotonated and the complex formation can be described by the reaction

$$\mathrm{Me}^{2+} + \mathrm{EDTA}^{4-} \rightleftharpoons \mathrm{MeEDTA}^{2-} \tag{a}$$

where Me denotes Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}.

The initial solution of EDTA was prepared from the acidic form of EDTA, and the pH was adjusted with tetramethylammonium hydroxide (TMAOH). Harada *et al.*(16) found an ultrasonic relaxation in Na^+ salts of EDTA at pH 12 resulted from the association reaction of Na^+ with EDTA. This process may result in a relaxation contribution to the compressibility of the solution and the ultrasonic velocity due to the pressure and temperature dependence of the binding constant. To avoid this contribution we then used TMA^+ salt of EDTA in our measurements. We found that this salt shows no excess ultrasonic absorption in the pH range 5 to 12 at 7 MHz, while for Na^+ salts of EDTA ultrasonic absorption increases at pH above 8 (see Fig. 1).

2.2. Methods

The resonator ultrasonic velocity and absorption measurements were done as previously described.(6,14,15,22) Titanium resonator cells of 0.8 ml volume, with built-in stirrers were used.(15) The resolution of measurements of $(U-U_0)/U_0$ was $10^{-4}\%$. The frequency was 7.1 MHz. The concentration increment of the ultrasonic velocity A was calculated from the equation:

$$A \equiv (U - U_0)/(U_0 m \rho_0) \tag{1}$$

where U and U_0 are the values of ultrasonic velocity in the solution and the solvent, respectively, m is the molal concentration of the solute, and ρ_0 is the density of the solvent. In the present work we used dilute aqueous solutions with concentration less then $0.01M$. For these concentrations the value of

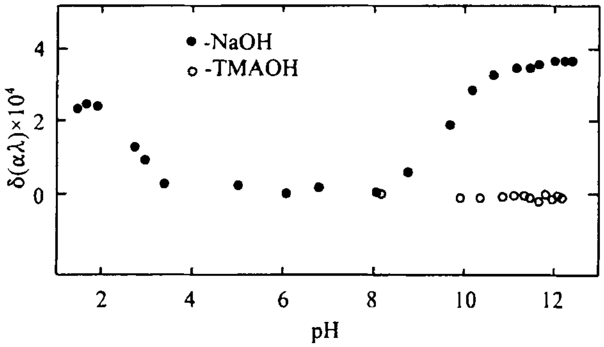

Fig. 1. Excess ultrasonic absorption per wavelength in the EDTA solution *vs.* pH during titration by NaOH and TMAOH at 25°C and at 7.1 MHz EDTA concentration, 0.003–0.004M.

ultrasonic velocity in our solutions differs by less then 0.1% from that for pure water. Therefore, within the resolution of our measurements the value of the ultrasonic velocity in pure water, $U(H_2O)$, can be used instead of U_0 in the denominator of Eq. (1). For the same reasons the density of pure water $\rho(H_2O)$ can be used instead of ρ_0. Another consequence of the low concentrations used in our measurements is the additivity of the contribution of solution components to the value of U for the solution.

The acoustic titrations were done, as earlier,(6) by adding defined amount of alkaline earth chloride solutions into the sample cell containing the EDTA solution. The reference cell contained water. Filling of the sample cell and adding of the titrant into the cell was done by Hamilton syringes with adaptors (Hamilton, Reno, U.S.).

The net effect of the complex formation is determined by the difference in the values of the ultrasonic velocity in the system MeEDTA and in the *unreacted* system (Me + EDTA), the right and left sides of Reaction (a). The ultrasonic velocity in the system MeEDTA was measured directly. The value of ultrasonic velocity in the system (Me + EDTA) was calculated from the concentration increment of the ultrasonic velocity of the TMA salt of EDTA and the concentration increment of the ultrasonic velocity of titrating salt in aqueous solution, assuming additivity. This implies the absence of any specific interaction between the salt and the other components of the buffer. One should emphasize that direct measurements of the ultrasonic velocity in the system (Me + EDTA) are difficult to make because of formation of Me^{2+} complexes with OH^- ions followed by aggregation. Even if these could be measured it would be impossible to evaluate the net effect of the MeEDTA complex formation because of the contribution of complexing of free Me^{2+} ions and OH^- ions. This contribution is absent in the MeEDTA system at the $[Me^{2+}]/[EDTA]$ ratio less then unity prevailing at our concentrations because of the high binding constant.(2,3) Finally the net effect of complex formation on the concentration increment of the ultrasonic velocity δA of EDTA

$$\delta A = [U(\mathrm{EDTA} + \mathrm{TMAOH} + \mathrm{MeCl_2}) - U(\mathrm{EDTA} + \mathrm{TMAOH}) - U(\mathrm{MeCl_2})]/[U(\mathrm{H_2O})\rho(\mathrm{H_2O})m] \qquad (2)$$

where $U(\mathrm{EDTA} + \mathrm{TMAOH} + \mathrm{MeCl_2})$ is the experimentally measured ultrasonic velocity in aqueous solution of EDTA, TMAOH, and titrating salt $MeCl_2$, $U(\mathrm{EDTA} + \mathrm{TMAOH})$ is the same value at zero concentration of titrating salt, $U(\mathrm{MeCl_2})$ is the ultrasonic velocity in aqueous solution of titrating salt, and m is the concentration of EDTA at each step of titration. The values of $U(\mathrm{MeCl_2})$ were calculated from the concentration dependence of the ultrasonic velocity in aqueous solutions of electrolytes.(13) Thus, the

δA value obtained is the change in concentration increment of ultrasonic velocity of EDTA in transferring Me^{2+} from the free state in solution into the complex with EDTA.

The excess sound absorption per wavelength, $\delta(\alpha\lambda)$, was measured as the difference in the attenuation coefficient per wavelength of EDTA solution and the buffer as described earlier.(14)

The densities of the solutions were measured using a densimeter (Model DMA-602, Anton Paar, Graz, Austria) with a total cell volume of 0.8 ml. The densimetric titrations of EDTA were realized using a vessel connected hermetically to the densimetric cell by pipes. At each step of the titration the solution was pumped out of the cell, mixed with a defined volume of $MeCl_2$ solution, and reinjected into the cell. The $MeCl_2$ solutions were added by Hamilton syringes with adaptors (Hamilton, Reno, U.S.) through a small hole in the stopper of the vessel. For calculations of apparent molar volume we used the well-known equation

$$V_\phi = M/\rho - D \tag{3}$$

where M is the molecular weight the EDTA, $D = (\rho - \rho_0)/\rho_0\rho m$ is the concentration increment of density, ρ is the density of the solution, and ρ_0 is the density of the pure solvent. The volume change in reaction (a), δV_ϕ, is given by

$$\delta(V_\phi) = \delta(M/\rho) - \delta D \tag{4}$$

The change in the density of solutions resulting from the binding of Me^{2+} ions to EDTA is less then 2×10^{-4} g-cm^{-3} at our concentrations of the EDTA. Therefore, the first term in Eq. (4) is less then 0.1 cm^3-mol^{-1}, *i.e.*, less than the experimental error in V_ϕ and can be neglected. The second term δD has the same structure as the δA value; therefore, an equation analogous to Eq. (2) was used to obtain it.

$$\begin{aligned}\delta D = {}& [\rho(\text{EDTA} + \text{TMAOH} + \text{MeCl}_2) - \rho(\text{EDTA} + \text{TMAOH}) \\ & - \rho(\text{MeCl}_2)]/[\rho(\text{H}_2\text{O})\rho(\text{H}_2\text{O})m]\end{aligned} \tag{5}$$

Here, the density values ρ are taken for the same solutions as the values of the ultrasonic velocities U in Eq. (2). Densities of the salt solutions were taken from the literature.(13,18)

Spectrophotometric titrations were carried out at 350 nm using a diode array spectrophotometer (Model 8452A, Hewlett Packard).

The change of concentration of EDTA in the acoustic, densimetric, and spectrophotometric titrations was less then 5%.

3. RESULTS

The results of the acoustic titrations of EDTA with Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} are shown in Fig. 2. The δA value decreases with increasing cation concentration. All curves have a break point at a ratio $[Me^{2+}]/[EDTA] = 1$, indicating the completion of the 1:1 complex formation. The small change of δA at $[Me^{2+}]/[EDTA] > 1$ for Ca^{2+}, Sr^{2+}, and Ba^{2+} ions can be explained by the formation of Me^{2+} complexes with OH^- ions possibly followed by aggregation. Even in the case of Mg^{2+}, a small increase of the slope of the ultrasonic titration curve is seen for $[Mg^{2+}]/[EDTA] > 1$. To investigate the contribution of aggregation to our titration curves spectrophotometric titrations of EDTA by Mg^{2+} were carried out under the same conditions as in the acoustic titrations. The resulting titration curve is given in Fig. 3. It is evident that aggregation starts only after the completion of the MgEDTA complex. Therefore, the changes of δA when $[Me^{2+}]/[EDTA] < 1$ can be attributed to the effects of complex formation alone.

The results of the density measurements are shown in Fig. 4. The densimetric titrations of EDTA with Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} were carried out at the concentration ratios $[Me^{2+}]/[EDTA]$ between 0 and 1, because the sound velocity data had shown that complexation is complete at $[Me^{2+}]/[EDTA] = 1$.

The *total* changes in the molar concentration increment of ultrasonic velocity, ΔA, the apparent molar adiabatic compressibility, $\Delta K_{s\phi}$, and the apparent molar volume, ΔV_{ϕ}, for the MeEDTA complex formation, Reaction

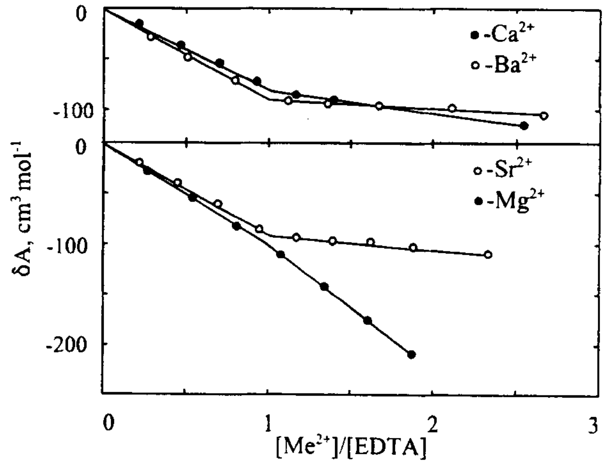

Fig. 2. Ultrasonic titration curves at 7.1 MH_2 of EDTA by $MeCl_2$ salts at 25°C and pH 12. EDTA concentration, 0.003–0.004*M*.

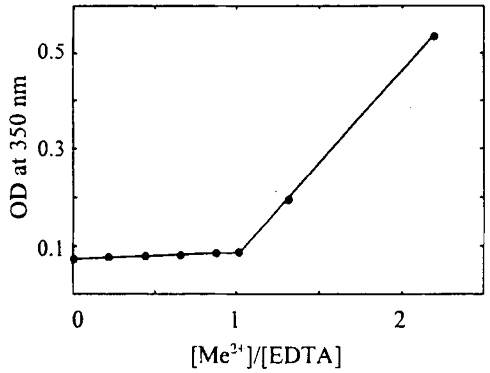

Fig. 3. Optical titration of EDTA by Mg^{2+} at 25°C and pH 12. EDTA concentration, 0.003–0.004*M*.

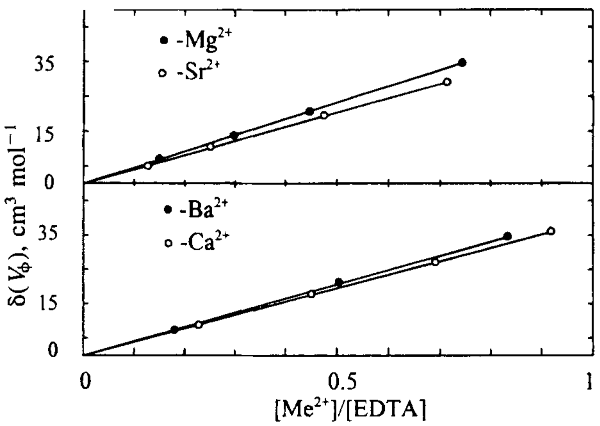

Fig. 4. Density titration curves of EDTA by cations at 25°C and pH 12. EDTA concentration 0.003–0.004*M*.

(a), are given in Table I. The change of apparent molar adiabatic compressibility, $\Delta K_{s\Phi}$, was calculated from ΔV_Φ and ΔA using the equation[19]

$$\Delta K_{s\Phi} = 2\beta_0(\Delta V_\Phi - \Delta A) \tag{6}$$

where β_0, is the coefficient of adiabatic compressibility of the solvent. Because of low concentrations of our solutions we used the coefficient of adiabatic compressibility of pure water instead of β_0 in the above equation. This value was calculated from data for the ultrasonic velocity U_0[20] and density ρ_0[21] of pure water using the well known equation

$$\beta_0 = 1/(U_0^2\rho_0) \tag{7}$$

Table I. Metal-EDTA Complex Formation at 25°C and pH 12: Effects on Ultrasonic Velocity, Apparent Molar Volume and Compressibility Parameters

Cation	ΔA^a	$\Delta V_\phi{}^a$	$10^4\Delta K_{s\phi}$	$10^4 K_{rel}{}^{b,d}$	$10^4\Delta(\Delta K_h)^b$	$10^{-4}k^c$	$10^4(\Delta K_h)_{Me}{}^{b,e}$
Mg^{2+}	−100.1±1	46.6±1.5	131.1±2.5	0	131.1	0.36	−63.4
Ca^{2+}	−77.9±1	39.6±1.5	105.0±2.5	1.1	103.9	0.38	−59.8
Sr^{2+}	−89.8±1	40.5±1.5	116.6±2.5	1.1	115.5	0.35	−75.9
Ba^{2+}	−94.1±1	41.4±1.5	121.0±2.5	3.4	117.6	0.34	−74.7

[a] cm^3-mol^{-1}.

[b] cm^3-mol^{-1}-bar^{-1}.

[c] Bar.

[d] Calculated from data of Ref. 16 by Eq. (12).

[e] $(\Delta K_h)_{Me} = K^o_{s\phi}$; the apparent molar adiabatic compressibility of the cation at infinite dilution (from Ref. 18).

4. DISCUSSION

4.1. Hydration Contributions $\Delta(\Delta V_h)$ and $\Delta(\Delta K_h)$ to the Volume and Compressibility Effects of Complex Formation

For dilute solutions the apparent molar adiabatic compressibility, $K_{s\phi}$, and the apparent molar volume, V_ϕ, are given by[6,7]

$$K_{s\phi} = K_m + K_{rel} + \Delta K_h \tag{8}$$

$$V_\phi = V_m + \Delta V_h \tag{9}$$

Here V_m is the intrinsic molar volume of the solute molecules that is inaccessible to the surrounding solvent; K_m is the molar compressibility of this volume; K_{rel} is the relaxation compressibility that represents the contributions of relaxation processes to the compressibility, ΔV_h is the hydration contribution to V_ϕ, *i.e.*, the volume change of the solvent around the solute molecules resulted from the solute–solvent interactions; ΔK_h is the hydration contribution to $K_{s\phi}$ resulting from the difference in compressibility of the hydration shell and bulk water.

The intrinsic volume V_m of simple molecules with no large voids in their structure, is mainly determined by the sum of van der Waals volumes of their atoms and atomic groups. In that case the intrinsic compressibility K_m is negligible.[22] Furthermore, in the present study only the changes of $K_{s\phi}$ and V_ϕ due to complex formation are considered. Therefore, the changes in the K_m and V_m values in the complex formation can be neglected in Eqs. (8, 9), and the hydration contributions to the volume and the adiabatic compressibility effects of EDTA-cation binding, $\Delta(\Delta V_h)$ and $\Delta(\Delta K_h)$, are given by

$$\Delta(\Delta K_h) = \Delta K_{s\phi} - K_{rel} \tag{10}$$

$$\Delta(\Delta V_h) = \Delta V_\phi \tag{11}$$

We estimated K_{rel} from the data of Harada *et al.*(16) These authors measured the ultrasonic relaxation absorption of EDTA complexes of Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} at pH 12 in the frequency range from 4.5 to 95 MHz. From this data we calculated the relaxation contributions to the molar increment of ultrasonic velocity A_{rel} and the apparent adiabatic compressibility K_{rel} using the equation(17)

$$A_{rel} = \frac{K_{rel}}{2\beta_0} = \frac{(\alpha\lambda)_{max}}{\pi m\rho[1 + (f/f_{rel})^2]} \tag{12}$$

where $(\alpha\lambda)_{max}$ is the maximum value of the excess ultrasonic absorption per wavelength, f_{rel} is the relaxation frequency, and f is the frequency at which the ultrasonic velocity is measured. The calculated K_{rel} values are given in Table I.

4.2. $\Delta(\Delta V_h)$ and $\Delta(\Delta K_h)$ Are Positive

The values of $\Delta(\Delta V_h)$ and $\Delta(\Delta K_h)$ obtained for the formation of MeEDTA complexes are positive (see Table I). This can be explained by a dehydration of the cations and the atomic groups of the EDTA, *i.e.*, by a transfer of water molecules from the hydration shell of the reaction partners into the bulk water. This explanation is consistent with the previous observations of the hydration of low molecular weight molecules. The compressibility and volume of water in the hydration shell of their charged and polar atomic groups is smaller than that of bulk water.(22–24)

The minimum number of water molecules released in the MeEDTA complex formation can be estimated from the $\Delta(\Delta K_h)$ values. Overall, the $\Delta(\Delta K_h)$ is determined by the number of water molecules released n and by the molar compressibility K_h of water in the hydration shell (molar compressibility of the hydration shell per water molecule): $\Delta(\Delta K_h) = n(K_h - K_w)$, where K_w is the molar compressibility of pure water. Evaluating the true K_h for the ions and EDTA atomic groups involved in the complex formation is difficult. The minimum value for K_h is zero, corresponding to incompressible water in the hydration shell. Therefore the minimum number of water molecules n_{min} released in the complex formation, is given by: $n_{min} = -\Delta(\Delta K_h)/K_w$. The K_w value at 25°C is 8.1×10^{-4} cm^3-mol^{-1}-bar^{-1} resulting in n_{min} in the range 13–16, depending on the ion.

4.3. Correlation Between the $\Delta(\Delta V_h)$ and $\Delta(\Delta K_h)$

Volume and compressibility changes in hydration processes are normally strongly correlated. This correlation is expressed as a ratio of volume and compressibility effects, $\Delta(\Delta V_h)/\Delta(\Delta K_h)$, or as a slope of the plot of partial molar volume at infinite dilution *vs.* partial molar compressibility at infinite dilution. Both values are usually denoted by k.(18,25,26) The k value is sensitive to the nature of atomic groups exposed to water. For different monovalent and divalent salts k is 0.36×10^4 bar;(25) for divalent cations k is 0.39×10^4.(18) Formation of divalent and trivalent ion pairs results in $k = 0.37\times10^4$.(25) For ionization of weak acids and bases that includes hydration of protons, k is 0.47×10^4.(26) For nucleic bases molecules whose hydrophobic atomic groups are exposed to water, k rises to 0.75×10^4.(22) For our MeEDTA complexes k is in the range 0.35×10^4 to 0.38×10^4 (see Table I), showing that the contribution from the dehydration of charged atomic groups in the MeEDTA complex formation dominates the observed volume and compressibility effects.

4.4. Structure of MeEDTA Complexes

$\Delta(\Delta K_h)$ of the MeEDTA complex formation can be formally separated into two contributions, *i.e.*, $\Delta(\Delta K_{hEDTA})$ for the dehydration of EDTA including the possible contribution from TMA^+ release, and $\Delta(\Delta K_{hMe})$, for the Me^{2+} cation.

$$\Delta(\Delta K_h) = \Delta(\Delta K_{hEDTA}) + \Delta(\Delta K_{hMe}) \qquad (13)$$

All Me^{2+} ions coordinate with the same atomic groups of EDTA and dehydrate the inner part of the cavity formed by EDTA.(31-36) Therefore, we may expect that the change in hydration of EDTA molecules contributing $\Delta(\Delta K_{hEDTA})$, is nearly the same for all Me^{2+} ions, and the change in the hydration of Me^{2+} cations is proportional to their hydration level ΔK_{hMe} in the free state: so that $\Delta(\Delta K_{hMe})$ can be replaced by $F\Delta K_{hMe}$

$$\Delta(\Delta K_h) = \Delta(\Delta K_{hEDTA}) - F\Delta K_{hMe} \qquad (14)$$

The factor F in this equation is the degree of dehydration of the cation in the complex and can vary from 0 to 1. $F = 0$ means no interaction between cations and EDTA, and $F = 1$ means full dehydration of cations. For ΔK_{hMe} values we used the apparent molar adiabatic compressibilities of ions at infinite dilution determined earlier by Lo Surdo and Millero(18) (see Table I).

In Fig. 5 $\Delta(\Delta K_h)$ are plotted against ΔK_{hMe}. Fitting of the data for Ca^{2+}, Sr^{2+}, and Ba^{2+} ions with Eq. (14) results in physically reasonable values of $\Delta(\Delta K_{hEDTA})$ and F, 56×10^{-4} cm^3-mol^{-1}-bar^{-1} and 0.8, respectively. The last

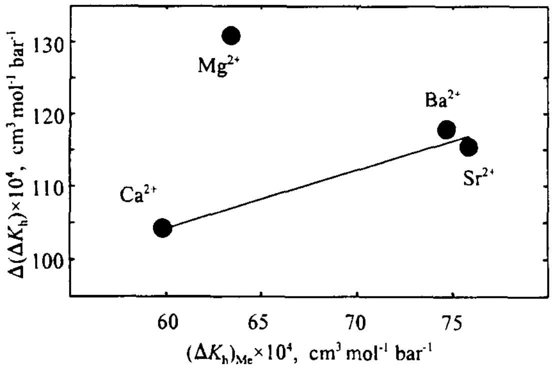

Fig. 5. Plot of $\Delta(\Delta K_h)$ of Me-EDTA complexes *vs.* ΔK_{hMe}.

value suggests that about 80% of the cation hydration shell is removed to the bulk water. Addition of the data for Mg^{2+}, the smallest ion, to the fit leads to unreasonable fitting parameters, *i.e.*, F, $\Delta(\Delta K_{hEDTA})$, and the correlation coefficient. The same is true for any combination of Mg^{2+} ion with two other ions. For the Mg^{2+} ion the compressibility effect of the complex formation with EDTA is much larger than the effects observed for the other cations. The MgEDTA complex seems to strip off a greater amount of hydration water. Such unusual behavior of Mg^{2+} can be attributed to the different binding mode to EDTA compared with other ions. The MgEDTA complex is packed in a hexacoordinated structure, with total dehydration of Mg^{2+}, while the complexes of Ca^{2+}, Sr^{2+}, and Ba^{2+} are mainly in pentacoordinated structures where water molecules are still coordinated to the cation and bound to the free coordination sites of the EDTA ligand.(16,27–38) Total dehydration of the Mg^{2+} ion assumes F to be equal to the one leading to $\Delta(\Delta K_{hEDTA}) = 67.7\times10^{-4}$ cm^3-mol^{-1}-bar^{-1}. This value is about 80% of $\Delta(\Delta K_{hEDTA})$ for Ca^{2+}, Sr^{2+}, and Ba^{2+} complexes. Finally it leads to the conclusion that both EDTA and the cation are 80% less dehydrated in the case of Ca^{2+}, Sr^{2+}, and Ba^{2+} complexes compared with the Mg^{2+} complex.

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