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INTERACTION OF CALCIUM ION WITH SODIUM TRIPHOSPHATE DETERMINED BY POTENTIOMETRIC AND CALORIMETRIC TECHNIQUES

SELWYN J. REHFELD AND HANS F. LOKEN
Department of Laboratory Medicine, University of California and the Veterans Hospital, San Francisco, Calif. 94143 (U. S. A.)
DELBERT J. EATOUGH
Thermochemical Institute, Brigham Young University, Provo, Utah 84602 (U. S. A.)
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ABSTRACT

The stoichiometry of the interaction of Ca^{2+} with sodium triphosphate was determined using a Ca^{2+} sensitive electrode, divalent ion sensitive electrode, a glass electrode and by titration calorimetry. A 2:1 and 1:1 complex of Ca^{2+} and $P_3O_{10}^{5-}$ is found when titrating calcium chloride with sodium triphosphate by the calcium ion sensitive electrode and tritration calorimetry. However, only by titration calorimetry is the 2:1 and 1:1 complex found when titrating sodium triphosphate with calcium chloride. Thermodynamic values (log *K*, Δ*H* and Δ*S*) are reported for the formation of $CaP_3O_{10}^{-3}$ and $Ca_2P_3O_{10}^{-}$ in aqueous solution.

INTRODUCTION

The development of the calcium and divalent ion selective electrodes[1] offers the possibility of their use in calcium binding studies in biological and detergent systems. Problems associated with adsorption of multicharged macromolecules such as proteins or surfactants on the electrode membrane often complicate the use of electrodes for the study of biological systems. Titration calorimetry may be more attractive for the study of such systems since the reponse of the sensing device, the thermistor, is unaffected by such interactions with the probe. For example, it has been recently shown that calorimetry may be used to study the interaction of proteins with erythrocyte membranes[2], of surfactants with proteins[3], and of alkali earth metal ions with proteins[4]. In addition, information on both the equilibrium constants and enthalpy changes associated with the reactions are often obtained in a calorimetric study. The purpose of this study is to evaluate potentiometric and calorimetric methods using as a model system the interaction of calcium chloride with sodium triphosphate.

EXPERIMENTAL PROCEDURE

Materials

Reagent grade $Na_5P_3O_{10}$ (Alfa Inorganics) and a standard aqueous $CaCl_2$ solution (Orion Research, Inc.) were used in this investigation. The $Na_5P_3O_{10}$ was further purified by repeated crystallizations from aqueous solutions of ethyl alcohol. Freshly distilled water was used in the preparation of all solutions. After preparation all solutions were stored under nitrogen.

Electrodes

An Orion calcium ion sensitive electrode (Model No. 92-20), an Orion divalent ion sensitive electrode (Model No. 93-32) and a Fischer combination glass electrode were used in this study. A single junction reference electrode (Orion Model No. 90-00-01) and a Corning Calomel electrode (fibre type No. 476000) were used as the reference electrodes. The calcium ion sensitive and divalent ion sensitive electrodes behaved in a completely Nernstien manner over the range of 10^{-1} to 10^{-5} M Ca^{2+} ion. However, the intercepts were not reproducible, in agreement with results previously reported by Rechnitz and Lin[5]. Thus, when using these electrodes, the response must be checked before and after each titration.

Calorimetric titrations

All runs were made on a Tronac Model 1000 isoperibol titration calorimeter similar to that previously described[6]. The method of operation and data evaluation have been previously described[7-9]. Runs were made at 25°C by titrating a 0.02 M $CaCl_2$ solution with $Na_5P_3O_{10}$ and by titrating a 0.02 M $Na_2P_3O_{10}$ solution with $CaCl_2$. Heats of dilution in both cases were determined by titration into distilled water.

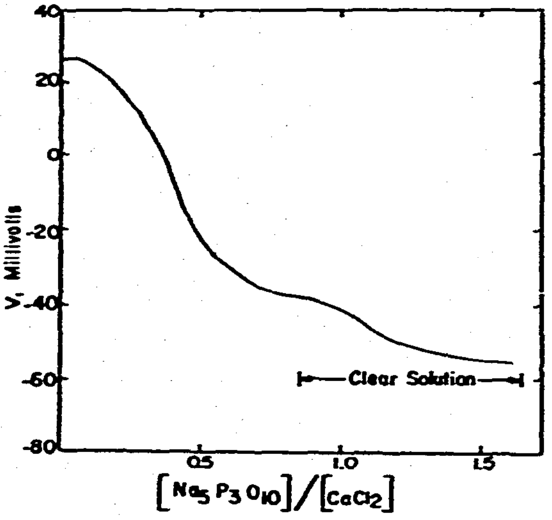
RESULTS

Calcium ion sensitive electrode

The calcium ion selective electrode response to the titration of a 0.0200 M $CaCl_2$ solution with 0.0308 M $Na_5P_3O_{10}$ solution is shown in Fig. 1. At very small $Na_5P_3O_{10}$:$CaCl_2$ ratios an initial rise in potential was observed. This rise in potential was reproducible, however, no explanation can be given at this time for this unusual response. Two inflection points are observed in this titration curve at Ca^{2+}:$P_3O_{10}^{5-}$ ratios of 1:1 and 2:1, respectively. However, the reverse titration curve could not be obtained using this electrode when the $Na_5P_3O_{10}$ solution was titrated with $CaCl_2$.

Divalent cation ion sensitive electrode

Titration of a 0.02 M $CaCl_2$ solution with 0.0308 M $Na_5P_3O_{10}$ solution using the divalent cation electrode gave only one inflection point at a Ca^{2+}:$P_3O_{10}^{5-}$ ratio of 2:1 as shown in Fig. 2. The response of the divalent cation sensitive electrode to the



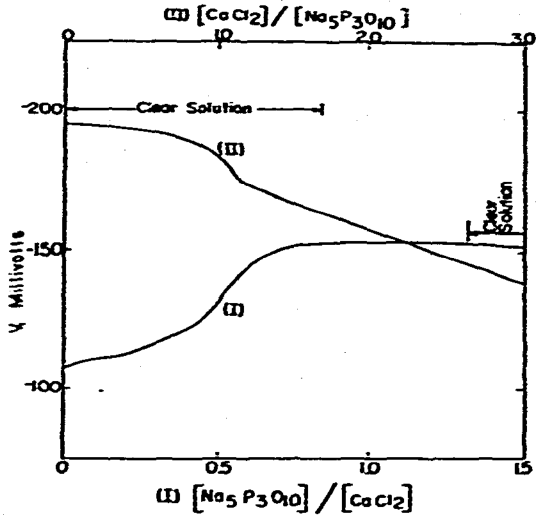
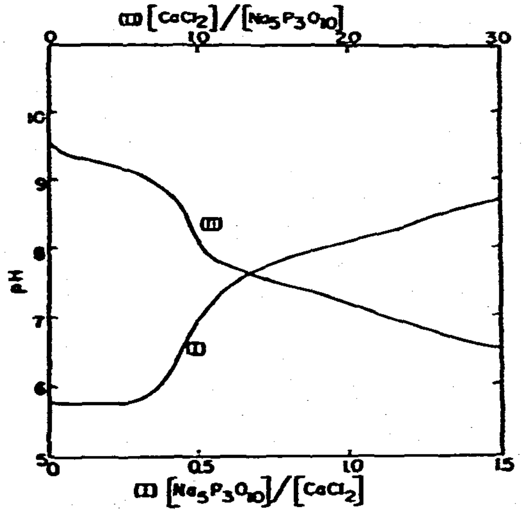


Fig. 1. Potentiometric titration curve for the titration of an aqueous solution of $CaCl_2$ with $Na_5P_3O_{10}$ at 25 °C using a calcium ion selective electrode.

Fig. 2. Potentiometric titration curves for the titration of aqueous solutions of: (I) $CaCl_2$ with $Na_5P_3O_{10}$; and (II) $Na_5P_3O_{10}$ with $CaCl_2$ at 25 °C using a divalent ion selective electrode.



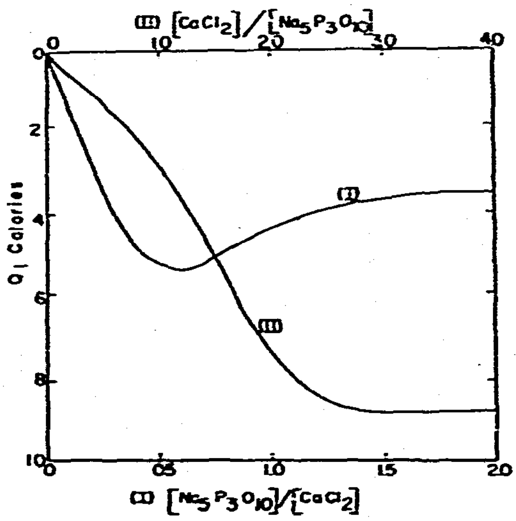


Fig. 3. The pH titration curves for the titration of aqueous solutions of: (I) $CaCl_2$ with $Na_5P_3O_{10}$; and (II) $Na_5P_3O_{10}$ with $CaCl_2$ at 25 °C using a glass electrode.

Fig. 4. The calorimetric titration curves for the titration of aqueous solutions of: (I) $CaCl_2$ with $Na_5P_3O_{10}$; and (II) $Na_5P_3O_{10}$ with $CaCl_2$ at 25 °C.

titration of $Na_5P_3O_{10}$ with $CaCl_2$ is also shown in Fig. 2. Again, only one inflection point is observed, but this time at a $Ca^{2+}:P_3O_{10}^{5-}$ ratio of 1:1.

Glass combination electrode

Titration of an aqueous solution of $CaCl_2$ with $Na_5P_3O_{10}$ using a glass combination electrode gave only one inflection point at a $Ca^{2+}:P_3O_{10}^{5-}$ ratio of 2:1 as shown in Fig. 3. Titration of $Na_5P_3O_{10}$ with $CaCl_2$ solution again gives only one inflection point, however, in this case it occurs at a $Ca^{2+}:P_3O_{10}^{5-}$ ratio of 1:1. These inflection points are the same as those reported for the divalent cation ion sensitive electrode.

Calorimetric studies

The interaction of $Na_5P_3O_{10}$ with $CaCl_2$ in aqueous solution was studied calorimetrically using both $CaCl_2$ and $Na_5P_3O_{10}$ as titrants. The heat liberated is shown in Fig. 4 for the titration of $CaCl_2$ with $Na_5P_3O_{10}$ (curve I) and $Na_5P_3O_{10}$ with $CaCl_2$ (curve II) at 25°C.

The calorimetric data show the existence of the two complexes containing Ca^{2+} and $P_3O_{10}^{5-}$ ions in the ratios 1:1 and 2:1 in both curve I and curve II. The titration data are best fitted assuming that the principal reactions occurring are:

$$Ca^{2+} + P_3O_{10}^{5-} = CaP_3O_{10}^{3-}; \qquad K_1, \Delta H_1^\circ \tag{1}$$

$$Ca^{2+} + CaP_3O_{10}^{3-} + Ca_2P_3O_{10}^{-}; \qquad K_2, \Delta H_2^\circ \tag{2}$$

$$Ca_2P_3O_{10}^{-} + Na^{+} = NaCa_2P_3O_{10}\,(\text{ppt}); \qquad K_p, \Delta H_p^\circ \tag{3}$$

A precipitate is formed in the titration given by curve I in the region from $[Na_5P_3O_{10}]/[CaCl_2] = 0.0$ to 0.5 and dissolves in the region from 0.5 to 1.0. The break in the titration curves at $R = 0.5$ corresponds exactly with the inflection points observed in the EMF titrations on the same systems. This indicates that the precipitate observed at low $P_3O_{10}^{5-}/Ca^{2+}$ ratios contains $2Ca^{2+}$ for every $P_3O_{10}^{5-}$, and is therefore either $NaCa_2P_3O_{10}$ or $HCa_2O_3P_{10}$. Previous literature results[10] report pK_4 and pK_5 values for $H_5P_3O_{10}$ to be 6.2 and 8.9, respectively. Using these pK values we calculate about 98% of the tripolyphosphate species present before reacting with the Ca^{2+} ion exists as $P_3O_{10}^{5-}$ ion under the conditions of the present study. The pH titration data given in Fig. 3 are consistent with this degree of hydrolysis. Also, previous studies[11,12] have indicated that Na^+ interacts with $P_3O_{10}^{5-}$ ion and the equilibrium constant for formation of the $NaP_3O_{10}^{4-}$ complex[12] may be as large as 370 l mol^{-1}. If the equilibrium constant for the $NaP_3O_{10}^{4-}$ species is this large then over 80% of the $P_3O_{10}^{5-}$ not complexed with Ca^{2+} will exist as the $NaP_3O_{10}^{4-}$ ion. Other Na^+ complexes would also be expected. We therefore conclude that the precipitate is most probably $NaCa_2P_3O_{10}$. This result is consistent with reported conductivity measurements[13].

Precipitate is present at the end of the titration given by curve II and the break in the titration curve at a Ca^{2+}:$P_3O_{10}^{5-}$ ratio of 2:1 again indicates the formation of a complex containing two Ca^{2+} ions for every $P_3O_{10}^{5-}$ ion. The fact that there is considerable curvature in the regions where $[Na_5P_3O_{10}]/[CaCl_2] = 0.5$ and 1.0 in curve I and where $[CaCl_2]/[Na_5P_3O_{10}] = 1.0$ and 2.0 in curve II indicates that the precipitate is not stoichiometrically formed in either case and that the equilibrium constants for the various reactions occurring in solution are such that all the species may be present in the solution concurrently.

The existence of appreciable $Ca_2P_3O_{10}^{-}$ ion in equilibria with the precipitate is suggested by curve II. The heat produced in the region where the Ca^{2+}:$P_3O_{10}^{5-}$ ratio is less that 1.0 is more exothermic than would be predicted if only $CaP_3O_{10}^{3-}$ and $NaCa_2P_3O_{10}$ (ppt) were present in appreciable concentrations. Assuming these are the only two species present in the titration, ΔH° values calculated from the inital slope and final Q values of curve I would predict that at least 3.7 cal of heat should be produced in this region, whereas only 2.8 cal are observed. We, therefore, conclude that reaction 2 contributes significantly to the titrations and that ΔH_2 is exothermic.

Log K and ΔH° values for reactions (2) and (3) have been calculated by a least squares technique[7-9] from the thermometric titration data. The value for log K_1 was taken from the literature[10,15,16] and a ΔH_1° value was calculated from the final Q value in curve I, at which point the only appreciable Ca^{2+} complex is the $CaP_3O_{10}^{3-}$ ion. Well-defined minima in the error square sum[7-9] were found for each titration curve; however, the K_2 and K_p values at the minima are different for the two curves. The values obtained for log K_2 and log K_p are 4.2 and 4.5 for curve I and 1.8 and 3.6 for curve II. Furthermore, the values of the error square sum at the minima are 0.4 and 3.8 cal^2 for curves I and II, respectively. If the log K and ΔH values described the titration curves within the accuracy of the calorimetric data, the magnitude of the error square sum at the minima would be less than 0.01 cal^2. The large difference in the log K values calculated from the two curves and the poor fit of the data in both cases indicates there are other reactions occurring in the calorimeter which contribute significantly to the species present and the heat produced. Most probably this involves interaction of Na^+ ion with the various negative ions present.

Interaction of Na^+ ion with the various species could be studied using a tetraalkyl ammonium tripolyphosphate. The K_p value is also small enough that the

TABLE 1

LOG K, ΔH AND ΔS VALUES FOR THE INTERACTION OF Ca^{2+} AND $P_3O_{10}^{5-}$ IN AQUEOUS SOLUTION AT 25°C

Reaction	*Log K*	*ΔH (kcal mol^{-1})*	*ΔS (gibbs mol^{-1})*
$Ca^{2+} + P_3O_{10}^{5-} = CaP_3O_{10}^{3-}$	6.4[a]	3.4	41
$Ca^{2+} + CaP_3O_{10}^{3-} = Ca_2P_3O_{10}^{-}$	3.0	−1.5	9
$Ca_2P_3O_{10}^{-} + Na^+ = NaCa_2P_3O_{10}$ (ppt)	4.0	8.0	45

[a] Taken from refs. 10, 15, and 16.

interactions could be studied in the absence of precipitate by using more dilute solutions.

The thermodynamic values which are presently considered to best describe the Ca^{2+} ion and $P_3O_{10}^{5-}$ interactions are given in Table 1. It should be remembered that in the calculation of these values both hydrolysis of the various aqueous species and any interaction of Na^+ ion with the tripolyphosphate species have been neglected. Therefore, these results should be considered as provisional values only.

DISCUSSION

A comparison of the species found in solution using each of the methods studied is given in Table 2. While species with Ca^{2+}:$P_3O_{10}^{5-}$ ratios of 1.0 and 2.0 were found by all techniques, only titration calorimetry clearly identified both species in titrations where $Na_5P_3O_{10}$ and $CaCl_2$ were titrants. This undoubtedly reflects the fact that the sensor used in calorimetry (i.e., the thermistor) does not employ a membrane sensing device and thus is not strongly affected by interactions at the detector surface. It can be expected that surface interaction problems with electrodes will become more serious in biological fluids and results obtained by such techniques should probably be verified by other analysis where possible.

TABLE 2

RATIO OF $CaCl_2$ TO $Na_5P_3O_{10}$ AT INFLECTION OR END POINTS WHEN TITRATING AQUEOUS $CaCl_2$ SOLUTIONS WITH AQUEOUS $Na_5P_3O_{10}$; AND, WHEN TITRATING AQUEOUS $Na_5P_3O_{10}$ SOLUTION WITH AQUEOUS $CaCl_2$, AT 25°C

System employed	*$CaCl_2$: $Na_5P_3O_{10}$*			
	Titration of $CaCl_2$ with $Na_5P_3O_{10}$		*Titration of $Na_5P_3O_{10}$ with $CaCl_2$*	
Calcium ion selective electrode	(1) 2.0	(2) 1.0	(1) —	(2) —
Divalent ion selective electrode	(1) —	(2) 1.0	(1) 2.0	(2) —
Combination glass electrode	(1) —	(2) 1.0	(1) 2.0	(2) —
Titration calorimetry	(1) 2.0	(2) 1.0	(1) 2.0	(2) 1.0

The two species reported in this study have not been identified in potentiometric[15–17], conductivity[13], solubility[18–20], nephelometry[14], or n.m.r.[21] studies. Reported log K values for the formation of the $CaP_3O_{10}^{3-}$ complex[10,14–20] vary from 1.7 to 6.8. The majority of the values, however, fall in the range from 5.0 to 6.8. The enthalpy change for formation of this complex (3.4 kcal mol^{-1}) determined calorimetrically in this study does not agree with those reported from the temperature dependence of the equilibrium constant (−3.2[14] and −6.6[22] kcal mol^{-1}) but is in good agreement with a reported[15] calorimetric study (3.3 kcal mol^{-1}). These earlier studies do not take into account formation of the 2:1 complex. Possibly much of the discrepancy in existing data would be improved if all species in solution were taken into account in the analysis of the data.

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