

Interaction of Alkaline Earth Metal Ions with Polyphosphates and Citrate in the Presence and Absence of Casein

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The ionization properties and complexing ability of pyro-, tripoly-, tetra-, and hexametaphosphate and of citrate for the four alkaline earth metal ions, calcium, magnesium, barium, and strontium were determined in the presence and absence of casein. The percentage ionization of the polyphosphates and citrate was measured with the Beckman specific ion electrode. The per-

centage dissociation and pH of the polyphosphates and citrate in 0.002M solutions was measured before and after addition of 1, 2, and 4 mmoles per liter of the four alkaline earth metal ions. Casein behaved as a multivalent cation and resulted in virtually complete displacement of sodium from the polyphosphates and citrate.

The chemistry of the condensed phosphates has been a subject of study for many years. In spite of their widespread industrial use, their ionic properties are relatively poorly understood. One of the major functions of the condensed phosphates is their ability to complex alkaline earth metal ions. This property is undoubtedly one of the major reasons for their use in food processing. The reactions of phosphoric acid polymers in biological systems are of equally great interest, and studies have been made of systems containing phosphatidic acid (1) and deoxyribonucleic acid (11).

There have been several studies on the complexing of alkali and alkaline earth metal ions by polyphosphates and citrate, and in some cases, the apparent dissociation constants of the complexes have been reported (4, 7, 9, 10, 13). The calculation of the apparent dissociation constants involves several assumptions; this makes the values obtained more useful for comparative purposes than for exact representation of the dissociation equilibrium. The reason for this difficulty is that the concentration of some of the ionic species can be determined only by indirect methods.

In previous work done in this laboratory (2), a specific ion electrode was used for the determination of sodium ion concentration. This method was extremely helpful and easy to perform, although the electrode measures sodium ion activity, which, in this paper and for comparative purposes, has been taken as a measure of concentration.

As there appears to be little information on the interaction of alkaline earth metal ions with polyphosphates and citrate in the presence of protein, and protein is usually present in biological systems where polyphosphate action is of interest, the authors decided to study this interaction in the presence and absence of casein. Casein can easily be obtained in a pure form and is a major constituent of milk, a system which is known to be affected greatly by polyphosphate addition.

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Experimental

The alkaline earth metal salts, trisodium citrate, and sodium pyrophosphate were reagent grade chemicals; sodium tripolyphosphate, tetrakisphosphate, and hexametaphosphate were commercial products.

Isoelectric casein was prepared by the method of El-Negoumy (6). The dialyzed product was freeze-dried and stored at 4° C. until used. The pH of a 3% suspension of this product in distilled water was 4.15 to 4.20.

Solutions of 0.002M polyphosphates and citrate were used to determine the effect of added alkaline earth metal ions on the dissociation of the sodium and the pH of the solution. In order to avoid changing total volume, the appropriate volumes of alkaline earth metal salt solutions were measured into beakers and the solutions dried. The polyphosphate and citrate solutions then were added to the dry residue in the beakers.

Sodium ion concentration was measured with a Beckman specific ion electrode for sodium in conjunction with a calomel reference electrode and a Metrohm Compensator Model E 322. This instrument was used also for the determination of pH. According to the technical information supplied by Beckman Instruments, Inc. (3), sodium ion concentration over the range 0.1 to 0.0001M should not be measured at pH values of less

Table I. Effect of Casein Addition on pH and the Millivolt Readings of Sodium Chloride Solutions^a

NaCl Mole per Liter	Unit	NaCl Solutions with pH Adjusted by TEA	NaCl Solutions with 3% Casein Added	
			pH not adjusted	pH adjusted by TEA
0.10	pH	9.77	4.20	8.10
	Mv.	0	0	0
0.010	pH	9.98	4.17	8.10
	Mv.	58.0	44.5	59.5
0.001	pH	9.92	4.16	8.05
	Mv.	115.0	64.5	115.5
0.0001	pH	9.94	4.15	8.10
	Mv.	171.0	65.5	163.5

^a Obtained with sodium ion electrode before and after adjustment of the pH to 8.0 with triethanolamine, with the value for the 0.1M solution taken as 0.

than 8 because the electrode is sensitive to hydrogen ions below this pH value also. The authors' work necessitated the use of the electrode at pH values below 8; therefore the effect of pH on the response of the sodium ion electrode was determined. Figure 1A gives the standard curves obtained with sodium chloride solutions adjusted with triethanolamine to pH values between 6.5 and 8.0. As the standard curves at the lower pH values were perfectly straight over the range 0.001 to 0.1M and only slightly displaced from the one obtained at pH below 8, the sodium ion concentration values obtained at a pH below 8 were calculated using this set of curves. All of the measured values in this work were between 0.003 and 0.1M. A similar procedure was used in the case of measurements in the presence of casein. The

effect of casein addition on the millivolt readings of sodium chloride solutions is given in Table I. As a result of the large influence of pH on the readings a set of measurements relating pH to millivolt readings was made and plotted as in Figure 1B, and sodium ion concentrations of solutions containing casein were obtained with these standard curves. When using the specific ion electrode, the absolute millivolt readings might differ slightly when standard solutions were measured at different times. However, in every case a straight line was obtained. When instead of using the absolute values relative millivolt values were used, there were no longer any variations with time. The millivolt readings used were those obtained by taking the value relative to that obtained for the 0.001M standard.

Table II. Dissociation of Sodium (%D) and pH of

	Concentration of Alkaline Earths Added, Mmoles per Liter	Unit	Added Salt				
			CaCl ₂	MgSO ₄	BaCl ₂	SrCl ₂	
Sodium pyrophosphate	0	%D ^b	65.1	65.1	65.1	65.1	
	1		81.2	81.2	75.0	73.7	
	2		86.2	86.2	78.1	81.2	
	4		95.0	90.0	86.2	86.2	
	0	%D ^a	89.0	89.0	89.0	89.0	
	1-4		100	100	100	100	
	0		pH ^b	9.89	9.89	9.89	9.89
	1			9.58	9.58	9.82	9.63
	2	9.17		8.48	9.79	9.44	
	4	7.48		7.61	9.42	7.58	
	0	pH ^a	5.90	5.90	5.90	5.90	
	1		5.43	5.35	5.59	5.70	
	2		5.29	5.06	5.31	5.44	
	4		5.17	4.92	4.85	5.29	
Sodium tripolyphosphate	0	%D ^b	61.0	61.0	61.0	61.0	
	1		69.0	69.0	69.0	70.5	
	2		76.5	75.0	72.0	78.0	
	4		81.0	81.0	76.0	84.5	
	0	%D ^a	92.0	92.0	92.0	92.0	
	1-4		100	100	100	100	
	0		pH ^b	9.66	9.66	9.66	9.66
	1			9.40	9.30	9.37	9.36
	2	8.41		7.95	9.15	8.81	
	4	7.44		7.32	8.30	7.81	
	0	pH ^a	5.90	5.90	5.90	5.90	
	1		5.43	5.24	5.65	5.72	
	2		5.36	5.01	5.30	5.47	
	4		5.15	4.90	5.08	5.28	
Sodium tetraphosphate	0	%D ^b	48.3	48.3	48.3	48.3	
	1		60.0	56.7	55.0	54.2	
	2		67.5	67.5	57.5	73.3	
	4		77.3	75.0	65.0	85.0	
	0	%D ^a	73.3	73.3	73.3	73.3	
	1-4		100	100	100	100	

^a In the presence of casein.

^b In the absence of casein.

From the known initial concentration of sodium and the sodium ion concentration as measured in each case the percentage dissociation (%*D*) was calculated.

Results

The effect of addition of 1, 2, and 4 mmoles per liter of the alkaline earth metal salts to 0.002*M* polyphosphate and citrate solutions is listed in Table II. In all cases the addition of increasing amounts of alkaline earth ions resulted in replacement of sodium bound to the polyphosphate or citrate complex by the alkaline earth metals. Invariably, the complex formation resulted in a release of hydrogen ions as evidenced by the drop in pH. The amount of complex formed depended on the levels

of alkaline earth metal ion added. The complexing ability of 0.002*M* solutions of the polyphosphates in the presence of 0.002 mole of the alkaline earth metal ions generally followed the sequence pyrophosphate > triphosphate > tetraphosphate > hexametaphosphate. The complexing ability of citrate at the 0.002*M* level was comparable to that of triphosphate. The behavior of citrate differed from that of the polyphosphates and showed the least effect of alkaline earth metal addition on the dissociation of the sodium, less than 10% *D* variation between 0 and 4 mmoles per liter addition, against as much as about 40% with some of the polyphosphates. There was also a smaller change in pH in the citrate solutions.

The differences in complexing ability, as indicated by

Solutions of 0.002*M* Polyphosphates and Citrate^a

	Concentration of Alkaline Earth Added, Mmoles per Liter	Unit	Added Salt				
			CaCl ₂	MgSO ₄	BaCl ₂	SrCl ₂	
Sodium tetraphosphate	0	pH ^b	8.39	8.39	8.39	8.39	
	1		7.85	7.60	8.04	7.99	
	2		7.13	6.89	7.49	7.37	
	4		6.31	6.26	6.67	6.49	
	0	pH ^a	5.87	5.87	5.87	5.87	
	1		5.48	5.28	5.50	5.57	
	2		5.21	5.07	5.08	5.41	
	4		4.95	4.35	4.97	5.14	
	Sodium hexametaphosphate	0	% <i>D</i> ^b	45.0	45.0	45.0	45.0
		1		50.0	46.7	47.9	48.3
2		58.7		58.7	58.8	61.2	
4		83.3		77.9	79.6	87.5	
0		% <i>D</i> ^a	65.4	65.4	65.4	65.4	
1-4			100	100	100	100	
0			pH ^b	7.45	7.45	7.45	7.45
1				7.01	6.76	7.04	6.97
2		6.43		6.27	6.67	6.51	
4		5.44		5.50	5.73	5.54	
0		pH ^a	5.87	5.87	5.87	5.87	
1			5.54	5.43	5.72	5.63	
2			5.49	5.26	5.50	5.52	
4			5.01	4.94	5.13	5.24	
Sodium citrate	0	% <i>D</i> ^b	69.5	69.5	69.5	69.5	
	1		70.0	76.7	69.5	69.5	
	2		76.7	78.3	75.0	75.0	
	4		78.3	79.2	76.5	76.5	
	0	% <i>D</i> ^a	95.0	95.0	95.0	95.0	
	1-4		100	100	100	100	
	0		pH ^b	7.48	7.48	7.48	7.48
	1			7.18	7.22	7.18	7.18
	2	6.81		6.82	6.95	6.84	
	4	6.42		6.41	6.63	6.53	
	0	pH ^a	5.71	5.71	5.71	5.71	
	1		5.01	4.84	5.10	5.17	
	2		4.98	4.75	4.99	5.07	
	4		4.80	4.69	4.88	5.02	

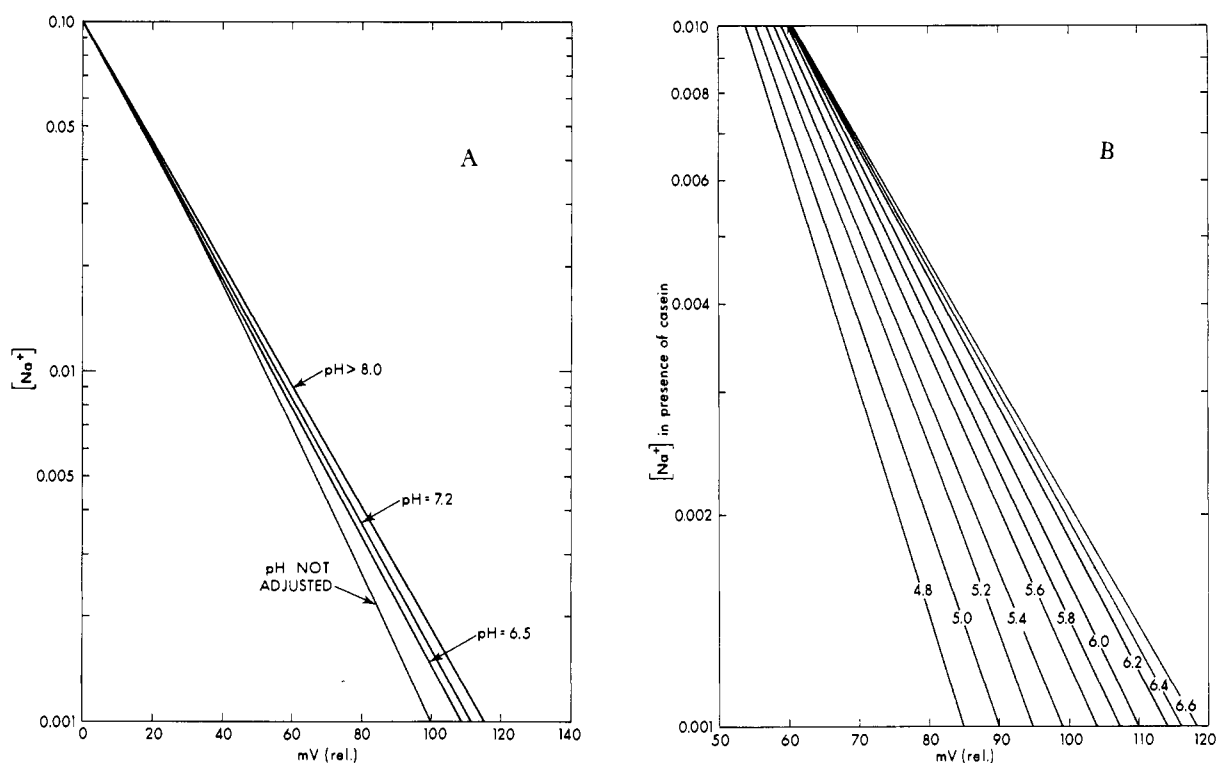


Figure 1. Standard curves for the determination of sodium ion concentration

Relative mv. readings obtained with the Beckman sodium ion electrode, *A*, before and after adjustment of the pH with triethanolamine and *B*, in the presence of casein, after adjustment of pH with triethanolamine

sodium ion replacement and drop in pH, of the polyphosphates and citrate for the four metal ions were relatively small. Pyrophosphate had a greater binding capacity for calcium and magnesium than for barium and strontium. Tripolyphosphate, tetraphosphate, and hexametaphosphate had about equal affinity for the metal ions, with the exception of the higher levels of strontium which were more effectively complexed. There were only minor differences in the complexing power of citrate for the four metal ions.

The effect of the presence of casein on the sodium dissociation and pH of polyphosphate and citrate solutions is also given in Table II. The sodium dissociation of all polyphosphates and citrate solutions was markedly increased by the presence of casein and without addition of alkaline earth metal ions (compare zero additions with those of Table II). After addition of 1 to 4 mmoles per liter of any of the alkaline earth metal ions the dissociation of the sodium was complete in all cases, the calculated %*D* values were between 99.6 and 102.2% and are reported as 100%. Apparently, the interaction of polyphosphates or citrate, casein, and alkaline earth metal ions resulted in a complete release of sodium from the polyphosphates and citrate.

Discussion

The four alkaline earth metal ions were similar in their ability to form complexes with the polyphosphates and citrate, and this resulted in the replacement of sodium and hydrogen ions from the ligands. The

effects of calcium and magnesium ions were similar in magnitude for removal of sodium as well as hydrogen ions. However, with barium considerably less hydrogen ion removal occurred, and this effect was most marked with pyrophosphate and became less as the chain length of the polyphosphates increased. With citrate there was only a small difference between the effect of barium on pH and the effect of the other ions.

Casein had a marked effect on the dissociation pattern of polyphosphates and citrate. In the presence of casein the dissociation of all of the polyphosphates and of citrate increased to completion with all of the levels of alkaline earth metal ions used. Apparently, casein behaves in a fashion similar to a multivalent cation and is very effective in replacing sodium and hydrogen ions from the polyphosphates and citrate.

A similar conclusion was reached in recent work by Lyons and Siebenthal (12), who found that strong binding occurred between calcium complexes and proteins. Pyrophosphate showed the strongest effect. Previous results from this laboratory (5) have indicated that pyrophosphate acts more strongly on protein systems than polyphosphates with longer chain lengths. This was evidenced by greater changes of color and viscosity of skim milk. The complexing effectiveness of the polyphosphates found in the present study compared on the basis of 0.002*M* solutions followed the sequence pyrophosphate > tripolyphosphate > tetraphosphate > hexametaphosphate. Callis and coworkers reported (9, 13) that the stability constant increases with increasing chain length; however, these studies were made at controlled

pH and differ from the present work where no pH adjustments were made. When polyphosphates are added to foods—e.g., milk—it is not common practice to readjust the pH and the changes occurring are usually small (5). In a previous study (5), deMan discovered that the interaction of polyphosphates and protein involves the binding of the calcium polyphosphate complex to the protein. More recent work has shown that the binding is quantitative. deMan also showed (5) that there is a marked dissimilarity in the interaction of polyphosphates and citrate with protein. As a result of the quantitative binding of the alkaline earth metal-polyphosphate complex to the protein, the concentration of soluble (ultrafilterable) calcium in the system decreased, whereas with citrate this concentration increased, because the citrate-calcium complexes are not quantitatively bound to the protein. However, in spite of the possible difference in the nature of interaction, polyphosphates and citrate react similarly when present in systems containing alkaline earth metals and protein. In all cases the protein seems to compete effectively for sites occupied by sodium or hydrogen and the effect is additive to that of the alkaline earth metal, resulting in a complete displacement of the sodium.

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