Complexometric Titration of Calcium and Magnesium in the Presence of Phosphate in Milk and Blood Plasma

T. H. KAMAL[1]
Department of Dairy Husbandry, University of Missouri, Columbia, Mo.

A quick and sensitive method for determining calcium and magnesium in biological materials, with particular reference to milk and blood plasma, is presented. The interference of phosphate ions in this complexometric titration was eliminated by adding the disodium salt of (ethylenedinitrilo)tetraacetic acid to the neutral system and back-titrating the excess with calcium and magnesium standard solutions. Milk and blood plasma samples were used directly without removing the phosphate ions or milk proteins.

The classical procedures for the determination of calcium and magnesium in biological materials are time-consuming and inefficient, especially when these elements are found in small amounts, as in blood plasma. There have been attempts to develop a rapid and accurate titration procedure based on the original work of Schwarzenbach and collaborators (*1*, *6*). The main difficulties encountered in analyzing biological materials are due to the interference of orthophosphate ions, which precipitate calcium and magnesium at the pH required for their determination.

These difficulties were overcome by removing phosphate ions by anion exchange resins and determining calcium in the effluent (*2*, *7*). Separation of calcium from phosphate ions by cation exchange and determination of calcium in the eluent were attempted in milk (*4*, *5*).

The application of ion exchange is, however, time-consuming, especially in milk samples where preremoval of proteins is necessary to avoid precipitation in the exchange column. Moreover, because of the several washings of the exchangers, the calcium and magnesium ions in the effluents or eluents become diluted and the end point of the titration becomes indistinct. This difficulty could be overcome in calcium determination by using a sensitive fluorescent indicator (*8*). The present study, however, determines the calcium and magnesium in milk and blood plasma directly by adding disodium salt of the (ethylenedinitrilo)tetraacetic acid to the neutral system and back-titrating the excess with calcium and magnesium standard solutions. Thus, it is not necessary to remove the milk proteins or use exchange resins to eliminate the interfering ions.

[1] Home address, Ministry of Supply, Cairo, Egypt.

Apparatus and Reagents

Magnetic stirring bars, Teflon coated.

Polyethylene-coated magnetic pickup rod.

Buffer Solution I, pH 13.0. Dissolve 560 grams of reagent grade potassium hydroxide and 66.0 grams of reagent grade potassium cyanide in 1 liter of glass-distilled water.

Buffer Solution II, pH 10.0. Dissolve 67.5 grams of C.P. ammonium chloride in 200 ml. of glass-distilled water, add 570 ml. of reagent grade concentrated ammonium hydroxide, and dilute to 1 liter.

Standard Calcium Solution, 0.1 and 0.0005%. Dissolve 2.4972 or 0.1249 gram of reagent grade calcium carbonate, previously dried at 110° C., in dilute hydrochloric acid. Make to 1 liter with glass-distilled water. This solution contains 1.00 mg. or 0.05 mg. of calcium, respectively, per ml.

Standard Magnesium Solution, 0.02 and 0.005%. Dissolve 0.2 or 0.05 gram of analytical reagent grade magnesium turnings in dilute hydrochloric acid and dilute to 1 liter with glass-distilled water. This solution contains 0.2 or 0.05 mg. of magnesium per ml.

Standard Solutions of (Ethylenedinitrilo)tetraacetic Acid Disodium Salt (Na_2EDTA), 1.5 and 0.06%. Dissolve 15 or 0.6 gram of the reagent in glass-distilled water and make to 1 liter. Standardize the solutions against the standard calcium and magnesium solutions. The titers for the two solutions are 1.607 or 0.0636 mg. of calcium per ml., and 0.968 or 0.0379 mg. of magnesium per ml., respectively.

Standard Phosphate Solution, 0.1%. Dissolve 4.3903 grams of C.P. potassium dihydrogen phosphate in glass-distilled water, and make to 1 liter. This solution contains 1.00 mg. of phosphorus per ml.

Calcium Indicator, calcein-charcoal–potassium chloride mixture manufactured by the G. Frederick Smith Chemical Co.

Magnesium Indicator. Dissolve 0.2 gram of Eriochrome Black T (Eastman Kodak P6361) in 50 ml. of analytical reagent grade methanol containing 2.0 grams of hydroxylamine hydrochloride.

Principle of Calcium and Magnesium Titration

Addition of excess Na_2EDTA to a neutral or slightly acidic solution containing calcium, magnesium, and phosphate ions causes chelation of calcium and magnesium with part of the Na_2EDTA, and thus prevents precipitation of calcium and magnesium as $Ca_3(PO_4)_2$ in alkaline medium. By back-titrating the excess Na_2EDTA with standard magnesium chloride solution at pH 10.0, and using Eriochrome Black T as indicator, the amount of Na_2EDTA chelated with the sum of calcium and magnesium ions can be calculated. On the other hand, adjusting another portion of the same solution to pH 12.0, after addition of excess Na_2EDTA, releases magnesium from its complex with Na_2EDTA, and the magnesium subsequently precipitates in the alkaline medium as magnesium hydroxide and/or phosphate. From the back-titration of the excess Na_2EDTA with standard calcium chloride solution at pH 12.0 to 14, and using calcein as indicator, one can calculate the amount of Na_2EDTA reacted with calcium. Subtracting the amount of Na_2EDTA reacted with calcium from that reacted with the sum of calcium and magnesium, one can obtain the amount of Na_2EDTA reacted with magnesium. By knowing the titer of Na_2EDTA for each of calcium and magnesium, the amount of calcium and magnesium in the solution can be determined.

Analytical Procedures

Calcium and Magnesium Determination in Milk. For calcium determination, 10 ml. of 1.5% Na_2EDTA are added to 5 ml. of milk in a 125-ml. Erlenmeyer flask and mixed thoroughly. Ten milli-

liters of buffer solution I are mixed with the sample. Then about 20 mg. of calcium indicator are added, which changes the color of solution from white to a light pink. The excess Na_2EDTA is back-titrated with 0.1% standard calcium chloride solution with gentle swirling, using a magnetic stirrer. At the end point, the pink color is changed to a green fluorescence. Dilution with water is avoided, and an artificial fluorescent light is used as background. The amount of Na_2EDTA reacted with calcium is calculated and multiplied by the titer for calcium to obtain the amount of calcium in the sample.

For magnesium determination, 10 ml. of 1.5% Na_2EDTA are added to 5 ml. of milk in a 125-ml. Erlenmeyer flask, and mixed thoroughly. Ten milliliters of buffer solution II are mixed with the sample, followed by 10 drops of Eriochrome Black T indicator just before the titration to minimize the loss of ammonia. The white color of the solution changes to a definite blue. The excess of Na_2EDTA is back-titrated with 0.02% standard magnesium solution, using a magnetic stirrer to obtain the amount of Na_2EDTA reached with the sum of calcium and magnesium. At the end point, the blue color is changed to a red-violet. Artificial fluorescent light is also used and dilution with water is avoided. The amount of Na_2EDTA reacted with calcium is subtracted from that reacted with the sum of calcium and magnesium. The result is multiplied by the titer for magnesium to obtain the amount of magnesium in the sample.

Calcium and Magnesium Determination in Blood Plasma. For calcium determination, 10 ml. of 0.06% of Na_2EDTA are added to 3 ml. of blood plasma in a 125-ml. Erlenmeyer flask, and mixed thoroughly. Five milliliters of buffer solution I are mixed with the sample and about 20 mg. of calcein indicator are added. The excess Na_2EDTA is back-titrated with 0.005% standard calcium chloride solution until the greenish yellow color of the sample is changed to a definite green fluorescence as an end point. Titration and stirring should be done slowly, to avoid foaming. An artificial light is used and dilution with water is avoided. The calculation is the same as that of milk calcium.

For magnesium determination 10 ml. of 0.06% Na_2EDTA are added to 3 ml. of blood plasma in a 125-ml. Erlenmeyer flask and mixed thoroughly. Five milliliters of buffer solution II are mixed with the sample, followed by 5 drops of Eriochrome Black T indicator, both added just before the titration. The excess Na_2EDTA is back-titrated with 0.005% standard magnesium chloride solution until the bluish color of the solution is changed to a blue-violet. Titration and stirring should be done slowly to avoid foaming. Precautions concerning the light and dilution as well as calculation are the same as in milk magnesium.

Results and Discussion

Ten-milliliter aliquots of 1.5% Na_2EDTA were pipetted into 125-ml. Erlenmeyer flasks, each containing 5.0 mg. of calcium and 1.0 mg. of magnesium. Increasing amounts of 0.1% standard phosphate solution were added to these flasks. Calcium and magnesium were analyzed by the procedures described above. Recovered amounts of calcium and magnesium are presented in Table I.

Recovery experiments for calcium and magnesium were also made using various milk and blood plasma samples. To each of the 5.0-ml. milk samples, previously analyzed for calcium and magnesium, 5.0 mg. of calcium, 2.0 mg. of magnesium, and 5.0 mg. of phosphorus were added. To each 3.0 ml. of blood plasma, 0.5 mg. of calcium, 0.5 of magnesium, and 0.5 mg. of phosphorus were added. The results of analyses, the amounts of calcium and magnesium recovered, and the standard deviations are presented in Table II for milk and blood plasma.

Table I shows that calcium and magnesium added either in the absence or presence of phosphate ions were recovered to a great extent by this method. Neither small nor large amounts of phosphate had any effect on the recov-

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Table I. Recovery of Calcium and Magnesium in the Presence of Phosphorus

Phosphorus, Mg. Added	Calcium, Mg.			Magnesium, Mg.		
	Added	Found[a]	Diff.	Added	Found[a]	Diff.
0.0	5.0	4.98	−0.02	1.0	1.02	+0.02
2.0	5.0	5.00	0.00	1.0	1.01	+0.01
3.0	5.0	4.95	−0.05	1.0	1.02	+0.02
5.0	5.0	5.03	+0.03	1.0	0.99	−0.01
8.0	5.0	4.95	−0.05	1.0	1.02	+0.02
10.0	5.0	4.98	−0.02	1.0	1.00	0.00

[a] Each value is an average of two independent determinations. Standard deviation for calcium ±0.0366. Accuracy ±0.732%. Standard deviation for magnesium ±0.0173. Accuracy ±1.73%.

Table II. Recovery of Calcium and Magnesium Added to Milk and Blood Plasma Containing Additional Phosphorus

Calcium, Mg.						Magnesium, Mg.					
	Added						Added				
Sample[a]	Ca	Mg	P	Found[a]	Diff.	Sample[a]	Ca	Mg.	P	Found[a]	Diff.
					Milk Samples						
5.014	5.0	2.0	5.0	10.108	+0.094	0.455	5.0	2.0	5.0	2.517	+0.062
5.769	5.0	2.0	5.0	10.831	+0.062	0.484	5.0	2.0	5.0	2.575	+0.091
5.560	5.0	2.0	5.0	10.606	+0.046	0.465	5.0	2.0	5.0	2.526	+0.061
5.062	5.0	2.0	5.0	10.156	+0.094	0.455	5.0	2.0	5.0	2.507	+0.052
5.030	5.0	2.0	5.0	10.060	+0.030	0.513	5.0	2.0	5.0	2.565	+0.052
5.432	5.0	2.0	5.0	10.462	+0.030	0.523	5.0	2.0	5.0	2.536	+0.013
4.982	5.0	2.0	5.0	10.060	+0.080	0.629	5.0	2.0	5.0	2.691	+0.062
					Blood Samples						
0.332	0.5	0.5	0.5	0.810	−0.022	0.034	0.5	0.5	0.5	0.534	0.000
0.292	0.5	0.5	0.5	0.806	+0.014	0.024	0.5	0.5	0.5	0.515	−0.009
0.295	0.5	0.5	0.5	0.791	−0.004	0.025	0.5	0.5	0.5	0.517	−0.008
0.332	0.5	0.5	0.5	0.820	−0.012	0.022	0.5	0.5	0.5	0.522	0.000
0.318	0.5	0.5	0.5	0.800	−0.018	0.023	0.5	0.5	0.5	0.534	+0.011
0.306	0.5	0.5	0.5	0.823	+0.017	0.033	0.5	0.5	0.5	0.527	−0.006
0.305	0.5	0.5	0.5	0.797	−0.008	0.006	0.5	0.5	0.5	0.497	−0.009

[a] Each value is an average of two independent determinations.
Standard deviation and accuracy for calcium ±0.073, ±1.46% in milk; ±0.016, ±3.20% in blood plasma.
Standard deviation and accuracy for magnesium ±0.065, ±3.25% in milk; ±0.008, ±1.60% in blood plasma.

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ery. Standard deviation was ± 0.0366 for calcium and ± 0.0173 for magnesium. In the presence of additional amounts of phosphate, the milk and plasma constituents do not seem to interfere with calcium and magnesium results (Table II). Calcium and magnesium in milk were accurate to ± 1.46 and ± 3.25%, respectively; to ± 3.20 and ± 1.60%, respectively, in blood plasma. The positive values of the recovery test shown in Table II for milk samples were a matter of chance in that set of determinations.

The color densities of end points are slightly different in milk than in plasma or blank solution because milk has a turbid background while plasma and blank solutions are transparent. However, at the end points a sharp change in color is observed.

Huditz, Flaschka, and Petzold (*3*) obtained reliable results by applying the principle of this investigation in the indirect determination of phosphorus. The excess of Na_2EDTA added to the acid solute of magnesium ammonium phosphate hexahydrate was back-titrated with magnesium standard solution. However, in calcium determination adding Na_2EDTA in excess to the solution and titrating the excess with a standard calcium chloride gave unreliable results (*5*). Malkki (*4*) later successfully used a similar method for calcium and magnesium determination in milk. However, with modifications in indicator, buffers, and reagent concentration, the method is applicable to both milk and blood plasma directly and with a high degree of accuracy.

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