Automated determination of Calcium and Magnesium in milk with 859 Titrotherm and 814 USB Sample Processor

Of interest to: Food (dairy) industry

Summary

Calcium and magnesium in milk can be rapidly and easily titrated thermometrically using a standard solution of Na_4EDTA as titrant. The milk sample is first treated with a trichloroacetic acid solution to coagulate protein and fat and liberate calcium and magnesium as dissociated ions. The serum containing the calcium and magnesium is separated by filtration or centrifugation. An aliquot of milk serum is treated with acetylacetone to enhance the separation of the Ca and Mg endpoints. The solution is then buffered with NH_3/NH_4Cl solution before titration.

This bulletin deals with the automated determination of calcium and magnesium (using an 814 USB Sample Processor) in commercially available finished milk products. Results are reported as mg/100 mL of Ca and Mg.

Introduction

In a titration, the titrant reacts with the analyte in the sample either exothermically (gives out heat) or endothermically (takes in heat). The Thermoprobe measures the temperature of the titrating solution. When all of the analyte in the sample has reacted with the titrant, the temperature of the solution will change, and the endpoint of the titration is revealed by an inflection in the temperature curve.

The amount of analyte determined is not related to the change in temperature of the solution. Therefore, it is not necessary to use insulated titration vessels.

Theory

Thermometric titrations are conducted under conditions of constant titrant addition rate. In this respect they differ from potentiometric titrations, where the titrant addition rate may be varied during the titration according to the electrode response. In thermometric titrations, a constant addition rate of titrant equates to a constant amount of heat being given out or consumed, and hence a more or less constant temperature change up to the endpoint.

Apparatus and accessories

1 x 2.859.1010 859 Titrotherm (1 Dosino and 1 Dosing unit 10 mL included)
1 x 2.814.0030 814 USB Sample Processor
2 x 2.800.0010 800 Dosino
1 x 6.3032.150 Dosing Unit 5 mL
1 x 6.3032.220 Dosing Unit 20 mL
1 x 6.1909.060 Stirring propeller (intensive)
22x 6.1459.300 PP sample tube 120 mL
1 x 6.9914.159 Titration head
1 x 6.2041.470 Sample rack 22 x 120 mL
3 x 6.1805.030 FEP tubing M6 150 cm
1 x 6.2061.010 Reagent organizer
1 x 6.2065.000 Stacking frame

Reagents

Solvent:	Deionized water
Standard:	$c(Mg^{2+})$ = 0.2 mol/L
Titrant:	$c(Na_4$ EDTA) = 1 mol/L
Buffer:	87.5 g NH_4Cl + 568 mL 28% (w/v) NH_3 solution made to 1000 mL with deionized water.
Reagent:	Acetylacetone
Coagulant:	Trichloroacetic acid 25% (w/v) in water

Samples

Full cream milk, pasteurized, homogenized
Low fat milk, pasteurized, homogenized
Skim milk, pasteurized, with added milk solids and milk calcium

Calculations

Molarity Na_4EDTA

The molarity of the titrant is computed from a regression analysis of titration results, where mmol of analyte (the standard) is plotted on the x-axis, and mL of titrant is plotted on the y-axis. This is computed automatically in ***tiamo***™, using the SLO command.

$c(Mg(NO_3)_2)$ mol/L= $c(Na_2HPO_4)$ mol/L/slope

Calculations for molarity in tiamo™

Assignment	RS name	Formula
RS01	EP1 mL	'EDTA titration.EP{1}.VOL'
RS02	Slope	'RS.EP1 mL.SLO'
RS03	Intercept	'RS.EP1 mL.ITC'
RS04	Corr Coeff	'RS.EP1 mL.COR'*'RS.EP1 mL.COR'
RS05	Molarity	'Std Mg solution.CONC'/'RS.Slope'
RS06	mmol Mg^{2+}	'MV.Sample size'*'Std Mg solution.CONC'
RS07	Titer	1/'RS.Slope.SLO'*'Std Mg solution.CONC'/'TET.CONC'

The value for the molarity of the standard magnesium solution is stored in Configuration>Titrants/

Solutions>Concentration against the relevant Dosing Unit.

The method *"Automated EDTA Standardization"* is available to perform this determination.

Calculations for method blanks

The method blank is determined by titrating different amounts of a representative product sample and plotting the sample amount against the titrant consumption. The method blank is determined as the y-intercept from a linear regression of the titration data. Changes in titrant dose rate or filter factor will require a new determination of the method blank. While a change in the titrant dose rate will require a new set of titrations to be run, a change in the filter factor can be performed by editing the set of titration data stored in the database.

This parameter is stored along with the other method parameters. For all sample determinations, the method blank in mL is subtracted from the volume of titrant.

In the case of this determination, it is necessary to compute two method blanks: one for the Ca endpoint (EP1) and one for the Mg endpoint (EP2). There is mutual interference between Ca and Mg during the titration, and this must be compensated.

To obtain an accurate estimate for the Mg blank, separate regression analyses are performed on EP1 and EP2, with the intercept for EP1 being subtracted from that for EP2.

Before performing a blank determination, it is important to set up in Configuration>Common Variables, CVs "Ca blank EDTA" and "Mg-Ca blank EDTA", as these CVs are used in the calculations for the determination of Ca and Mg in milk. These CVs can be entered automatically during the blank determination titration run by double clicking on the lines for "Intercept Ca" and "Corr blank Mg", selecting the "Options" tab, and checking the box "Save result as common variable", then selecting the correct CV title.

Calculation for method blank in tiamo™

Assignment	RS name	Formula
RS01	EP1 mL	'EDTA titration.EP{1}.VOL'
RS02	Slope Ca	"RS.EP1 mL.SLO'
RS03	Intercept Ca[1]	'RS.EP1 mL.ITC'
RS04	Corr Coeff Ca	'RS.EP1 mL.COR'*'RS.EP1 mL.COR'
RS05	EP2 mL	'EDTA titration.EP{2}.VOL'
RS06	Slope Mg	"RS.EP2 mL.SLO'
RS07	Intercept Mg	'RS.EP2 mL.ITC'
RS08	Corr Coeff Mg	'RS.EP2 mL.COR'*'RS.EP2 mL.COR'
RS09	Corr blank Mg^{2+}	'RS.Intercept Mg'-'RS.Intercept Ca'

[1] *"Intercept Ca" = Ca blank*
[2] *"Corr blank Mg" = corrected Mg blank*

The method *"Automated determination of Ca & Mg blanks-milkTCA"* is available to perform this determination.

Calculations for calcium and magnesium determinations in tiamo™

Assignment	RS name	Formula
RS01	EP1 mL	'EDTA titration.EP{1}.VOL'
RS10	Ca mg/100 mL	(('RS.EP1 mL'-'CV.Ca blank EDTA')*'EDTA titration.CONC'*40.078*100)/'MV.Sample size'
RS11	Mean Ca mg/100 mL	'RS.Ca mg/100 mL.MNV'
RS12	Std Dev Ca mg/100 mL	'RS.Ca mg/100 mL.ASD'
RS05	EP2 mL	'EDTA titration.EP{2}.VOL'
RS13	Mg mg/100 mL	(('RS.EP2 mL'-'CV.Mg-Ca blank EDTA')*'EDTA titration.CONC'*24.305*100)/'MV.Sample size'
RS14	Mean Mg mg/100 mL	'RS.Mg mg/100 mL.MNV'
RS15	Std Dev Mg mg/100 mL	'RS.Mg mg/100 mL.ASD'

Note: Where only single titrations on a sample are performed, lines for mean and standard deviation values may be omitted.

Methods

Procedure for determination of titrant molarity

Use the method "*Automated EDTA Standardization*". Set up a 20 mL Dosino to dispense the c(Mg^{2+}) = 0.2 mol/L standard. In Workplace>Run>Determination Series create a sample table with 6 sample positions. Enter the method "Automated EDTA standardization" in each position. In sample positions 1-6, enter values of 20, 18, 15, 12, 9 and 6 (mL) respectively. Save the sample table as "EDTA standardization with 0…2 mol/L Mg". Check the statistics box, and set the number of samples to 6.

Place two tubes filled with approx. 100 mL of deion. water in positions 21 and 22 of the sample rack. These are intended for the "double dip" rinse of the titration assembly after each titration.

Set up sample tubes as follows in positions 1…6 of the sample rack:

Sample rack position	Approx. mL deion. water
1	0
2	2
3	5
4	8
5	11
6	14

NOTE: It is essential that the titration assembly must be so adjusted that the propeller stirrer is ~1 mm above the bottom of the titration tube at the fully lowered position. The Thermoprobe and the fluid delivery tubes should be 1 mm above the tips of the propeller. It is recommended that the fluid delivery tubes be tied together with a plastic tie about 35 mm from the bottom, and the group of delivery tips angled towards the Thermoprobe. To minimize noise, the direction of the stirrer rotation must be set to carry the delivered fluid away from the sensor of the Thermoprobe. This setup should be used for all automated determinations.

Procedure for method blank determination

A method blank for the type of sample under examination is determined by titrating a range of sample amounts and calculating the y-intercept (in mL) of a regression curve formed by plotting sample amount (x-axis) against mL of titrant delivery (y-axis). This can be done automatically in ***tiamo***™.

Prepare a quantity of milk serum by transferring 500 mL of milk product into a 1000 mL beaker equipped with a large magnetic spin bar. Place on a magnetic stirrer, and while stirring, add 50 mL 25% (w/v) trichloroacetic acid slowly. Allow to stir for 10 minutes. Either filter the coagulated milk through a Whatman no. 4 filter paper (or similar), or separate the milk serum by centrifugation. Approximately 200 mL of milk serum is required.

Place two tubes filled with approx. 100 mL of water in positions 21 and 22 of the sample rack. These are intended for the "double dip" rinse of the titration assembly after each titration.

Prepare a series of titration tubes as follows:

Rack position	[1] Milk serum aliquot, mL	Deion. water, mL
1	50	0
2	40	10
3	30	20
4	25	25
5	20	30

[1] *Aliquots are to be dispensed by glass volumetric (bulb) pipettes.*

To each tube, add (by air pipettes) 800 µL acetylacetone. Before titration with Na_4EDTA, 5 mL of NH_3/NH_4Cl buffer is added automatically by a Dosino.

In Workplace>Run>Determination Series, prepare a sample table for 5 sample positions, with the method "*Automated determination of Ca & Mg blanks-milkTCA"* in each position, and the sample size varied to match the rack position in the above table. Check the „Statistics" box, and nominate 5 samples to be titrated.

Procedure for automated determination of calcium and magnesium in milk products

Prepare an aliquot of serum of the milk product by pipetting 100 mL of milk into a 250 mL beaker equipped with a large magnetic spin bar. Set on a magnetic stirrer, and slowly add by bulb pipette 10 mL 25% (w/v) trichloroacetic acid solution. Allow to stir for 10 minutes. Separate the clarified milk serum either by filtration through a Whatman no. 4 filter paper or by centrifugation. Pipette 50 mL of milk serum into a titration tube and add 800 µL acetylacetone. Before titration with Na_4EDTA, 5 mL of NH_3/NH_4Cl buffer is added automatically by a Dosino.

Place two tubes filled with approx. 100 mL of water in positions 21 and 22 of the sample rack. These are intended for the "double dip" rinse of the titration assembly after each titration.

In Workplace>Run>Determination Series, prepare a sample table for the appropriate number of sample positions, with the method "*Automated determination of Ca & Mg-milkTCA"* in each position. Nominate a sample size of 45.45 mL, and identify each milk product according to its sample position. Note that a 50 mL aliquot is equivalent to 45.45 mL of original milk sample, since the addition of trichloroacetic acid solution needs to be taken into account.

Results

Molarity of Na_4EDTA titrant

Slope	0.2000
Intercept, mL	0.0642
Correlation (R^2)	1.0000
Molarity, mol/L	1.0030
Titrant dose rate, mL/min	4
Filter factor	25

Blank determinations

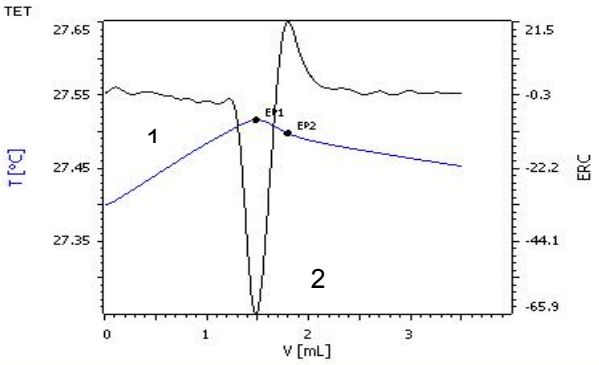
	Full cream milk	Low fat milk
Ca slope	0.0284	0.0331
Ca intercept, mL	0.0623	0.0547
Ca Correlation (R^2)	0.9999	0.9999
Mg slope	0.0335	0.0388
Mg intercept, mL	0.1320	0.1329
Mg Correlation (R^2)	0.9998	0.9999
Corrected Mg blank	0.0697	0.0782
Titrant dose rate, mL/min	4	4
Filter factor	15	15

	Skim milk with milk solids and milk calcium
Ca slope	0.0471
Ca intercept, mL	0.0353
Ca Correlation (R^2)	1.0000
Mg slope	0.0526
Mg intercept, mL	0.1626
Mg Correlation (R^2)	0.9998
Corrected Mg blank	0.1274
Titrant dose rate, mL/min	4
Filter factor	15

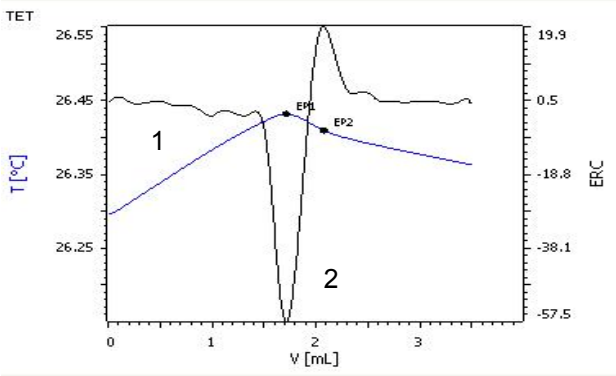
Results of calcium and magnesium analysis

	Full cream milk	Low fat milk	Skim milk with milk solids and milk calcium
Nutrition Information on label Ca mg/100 mL	117	145	175
Titrotherm Ca mg/100 mL	125.9±0.50 (n=5)	147.0±0.44 (n=9)	209.7±0.45 (n=8)
Titrotherm Mg mg/100 mL	13.2±0.41 (n=5)	14.8±0.59 (n=9)	14.5±0.33 (n=9)

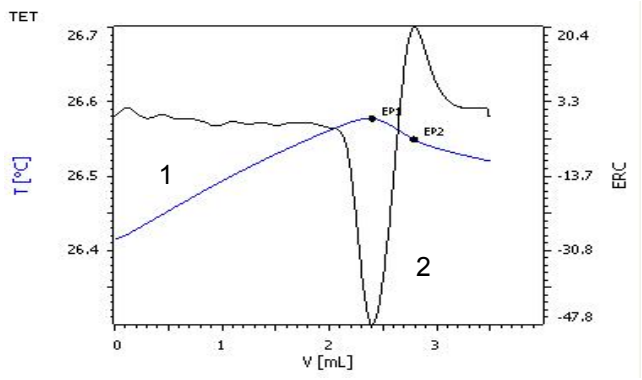
Titration plots



Ca and Mg in full cream milk



Ca and Mg in low fat milk



Ca and Mg in skim milk with added milk solids and milk calcium

Notes

1 = solution temperature
2 = second derivative curve (for endpoints)
EP1 = calcium endpoint (exothermic reaction, negative endpoint "peak")
EP2 = magnesium endpoint (endothermic reaction, positive endpoint "peak")

Notes on safe usage and disposal of trichloroacetic acid.

CCl_3COOH (trichloroacetic acid) and its solutions are toxic and corrosive. Wear appropriate protective clothing. Adhere to recommendations in MSDS documentation. Disposal of CCl_3COOH solutions and residues containing CCl_3COOH should be in accordance with local regulations.