Thermo. Titr. Application Note No. H-103

Title:	Determination of Calcium and Magnesium in Milk

***Scope*:**	Determination of calcium and magnesium in milk.

***Principle*:**	A measured amount of milk is treated with trichloroacetic acid to coagulate milk solids and liberate calcium and magnesium as dissociated ion. The coagulated milk is filtered or centrifuged, and an aliquot of the clear serum is titrated with a standard solution of 1mol/L tetra-sodium EDTA to thermometrically determined endpoints for Ca and Mg. Acetylacetone is added to alter the Ca- and Mg-EDTA stability constants for better endpoint sharpness. $Ca^{2+} + Y^{4-} \leftrightarrow CaY^{2-}$ (Y = EDTA) $Mg^{2+} + Y^{4-} \leftrightarrow MgY^{2-}$ **Thermodynamic Constants:** Heat of chelation, Ca^{2+} with EDTA: $\Delta H_r \approx$ -23.4 KJ/mol Heat of chelation, Mg^{2+} with EDTA: $\Delta H_r \approx$ +20.1 KJ/mol *The reaction with Ca^{2+} is thus exothermic, and that of Mg^{2+} endothermic.* **Stability Constants of EDTA complexes (as log K):** Ca^{2+} = 10.7, Mg^{2+} = 8.7

Reagents:	3.1. c(Na_4EDTA)=1mol/L. Prepare from A.R. tetrasodium EDTA. Alternatively, weigh 372.24 g A.R. Na_2H_2EDTA and quantitatively transfer to a 1000mL volumetric flask. 80g A.R. NaOH is carefully dissolved in 500mL D.I. water, cooled, and added to the same flask. When all solids have dissolved (with the addition of more water), make to volume and mix well. The EDTA solution may be standardized against a c(Mg^{2+}) = 0.2 mol/Lsolution, prepared from Mg metal according to AN H-075. 3.2. NH_3/NH_4Cl buffer. Dissolve 17.5 g A.R. NH_4Cl in 172 mL A.R. conc. (28%) NH_3 soln. and make to 250 mL with deionised water. 3.3. Acetylacetone (2, 4-Pentanedione). CAS 123-54-6, Aldrich cat. no. P7754. 3.4. Trichloroacetic acid, 25% w/v in D.I. water

Method:	*Basic Experimental Parameters:*	
	Titrant delivery rate (mL/min.)	4
	ERC Ca EP	-15
	ERC Mg EP	+5
	Data smoothing factor (DSF)	15
	Stirring speed (802 stirrer)	15
	Delay before start of titration (secs.)	10
	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 soluton. Allow to stir for 10 minutes. Separate 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 vessel and add 800 µL acetylacetone. Prior to the titration with Na_4EDTA, 5 mL of NH_3/NH_4Cl buffer is added automatically by Dosino. The volume of original milk contained in a 50 mL aliquot delivered for titration is 100*50/(100+10)=45.4545 mL	

		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)

Calculations:	
	Ca mg/100 mL =((EP1-Ca blank)*M EDTA*40.078*100)/sample vol., mL Mg mg/100 mL = ((EP2-EP1-Mg blank)*M EDTA*24.305*100)/sample vol, mL

Determination of Ca and Mg blanks

The method blank is determined by titrating different amounts of a representative sample of the product 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.

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 correctly.

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.

Examples of Ca and Mg blank estimations:

	Full cream milk	*Low fat milk*	*Skim milk with milk solids and milk calcium*
Ca intercept (blank), mL (1)	0.0623	0.0547	0.0353
Mg intercept, mL (2)	0.1320	0.1329	0.1626
Corrected Mg blank (2)-(1)	0.0697	0.0782	0.1274

Examples of titration plots

Blue curve = solution temperature
Black curve = second derivative of solution temperature
EP1 = calcium endpoint (exothermic reaction, negative endpoint "peak")
EP2 = magnesium endpoint (endothermic reaction, positive endpoint "peak")

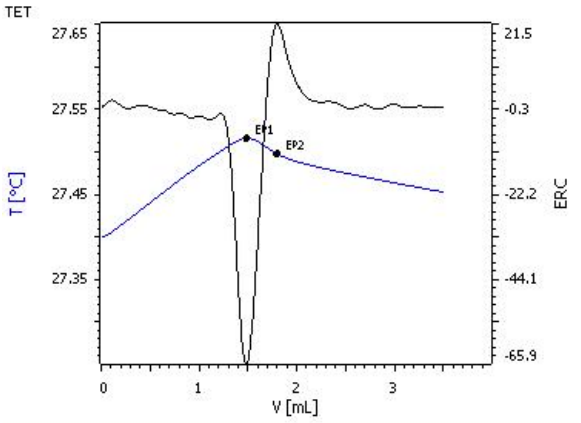

Fig. 1. Ca and Mg in full cream milk

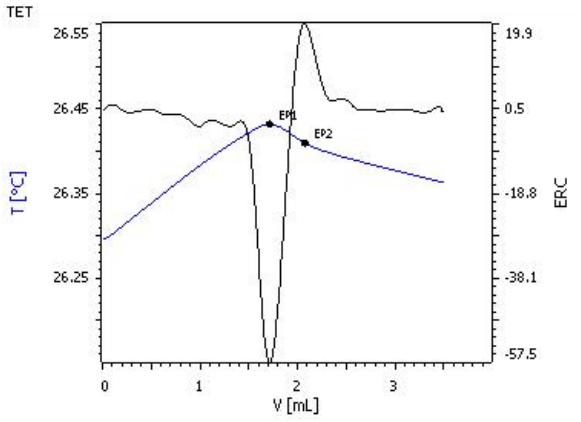

Fig. 2. Ca and Mg in low fat milk

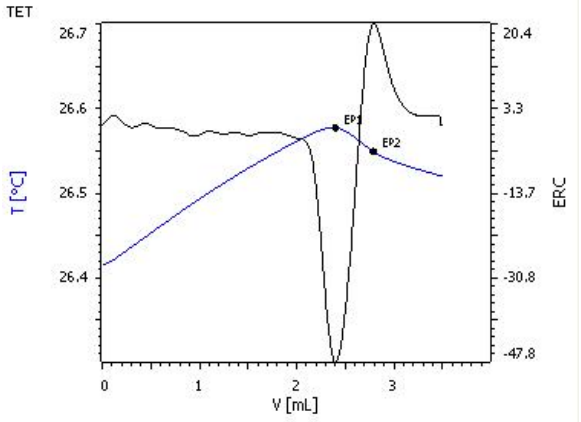

Fig. 3. Ca and Mg in skim milk with added milk solids and milk calcium

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.