

SOUTHWESTERN RADIOLOGICAL HEALTH LABORATORY

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DETERMINATION OF STRONTIUM-89 AND STRONTIUM-90 IN WHOLE MILK

PRECIPITATION AND SEPARATION OF MILK
PROTEIN BY TRICHLOROACETIC ACID

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SUMMARY

The precipitation and separation of protein from milk by trichloroacetic acid (TCA) was examined to obtain optimum conditions for strontium recovery. The variables under consideration were: TCA concentration, TCA addition rate, extent of mixing required, solution temperatures, and the effect of freezing the milk sample.

It was found that the present method¹ meets most of the requirements for maximum strontium yield if the procedure is followed strictly. The one improvement which might be made is in pre-heating the milk to speed up the filtration step.

Additional information was obtained in the form of a pH curve which indicated that approximately 50 ml of the TCA was lost after filtration.

PURPOSE

The purpose of this work is to minimize the strontium loss across the TCA precipitation step without overcomplicating the procedure.

EXPERIMENTAL

The first step in a routine analysis¹ of milk for radio-strontium is the precipitation (denaturation) of the milk protein by trichloroacetic acid (TCA). The protein solids may then be filtered off leaving the desired strontium in the filtrate liquor.

The effects of the following conditions were studied with respect to the strontium recovery and ease of separation.

- I TCA concentration
- II TCA addition rate
- III Temperature of TCA and milk solutions
- IV Extent of mixing required
- V Previous freezing of milk

Because of its greater abundance in milk and its chemical similarity to strontium, the calcium recovery was determined instead of the recovery of strontium.

Calcium was determined by titrating aliquots of the sample with EDTA² followed by back titration with standard calcium solution.

VI As a means of determining the amount of TCA lost in the precipitation step, a pH curve was made showing the pH of different TCA concentrations. The approximate TCA concentration of a sample was determined by checking its pH against the curve.

APPARATUS

Filter apparatus, 19 cm. Buchner funnel, 3 liter suction flask.

pH meter, Beckman zeromatic.

REAGENTS

Trichloroacetic acid (TCA), 50% W/V, 500 g TCA made up to 1 liter with distilled H₂O.

Milk, ten 1/2 gallon containers of Anderson Dairy homogenized milk were mixed and preserved with 50 ml of 37% formaldehyde.

PROCEDURE

I. To five 1000 ml aliquots of milk were added 100, 200, 300, 400, and 500 ml of 50% TCA. After thorough stirring with a glass rod the samples were filtered through a Whatman number 2 filter paper in a Buchner funnel. The precipitates were washed with approximately 200 ml distilled water and the total filtration and washing time for each sample was noted. The filtrates obtained were made up to 2000 ml with distilled water. Aliquots of this solution were used for the calcium determination.

II. To three 1000 ml aliquots of milk were added 300 ml portions of TCA. The TCA was added rapidly to the first sample with vigorous hand stirring. The TCA was added slowly to the second sample with vigorous stirring, allowing approximately 5 minutes for the addition. A separatory funnel was used to add the TCA dropwise to the third sample, allowing about 15 minutes for this addition. A mechanical stirrer was used to mix the third sample during TCA addition.

The samples were then filtered, washed, and made up to volume as in I.

III. Three 1000 ml aliquots of milk were heated to 20°C, 40°C, and 80°C respectively. To these samples were added 300 ml portions of TCA that had been heated to matching temperatures.

The samples were then stirred by hand, filtered, washed, and made up to volume.

IV. After addition of 300 ml TCA, three samples were treated as follows:

1. The first sample was stirred lightly by hand and filtered.
2. The second was stirred vigorously for about 5 minutes before filtering.
3. Sample number 3 was stirred mechanically for about 15 minutes followed by filtering.

These samples were washed and brought up to volume as before.

V. A 1000 ml milk sample was frozen, thawed out, and then precipitated with 300 ml TCA. The solution was then filtered, washed, and made up to 2000 ml.

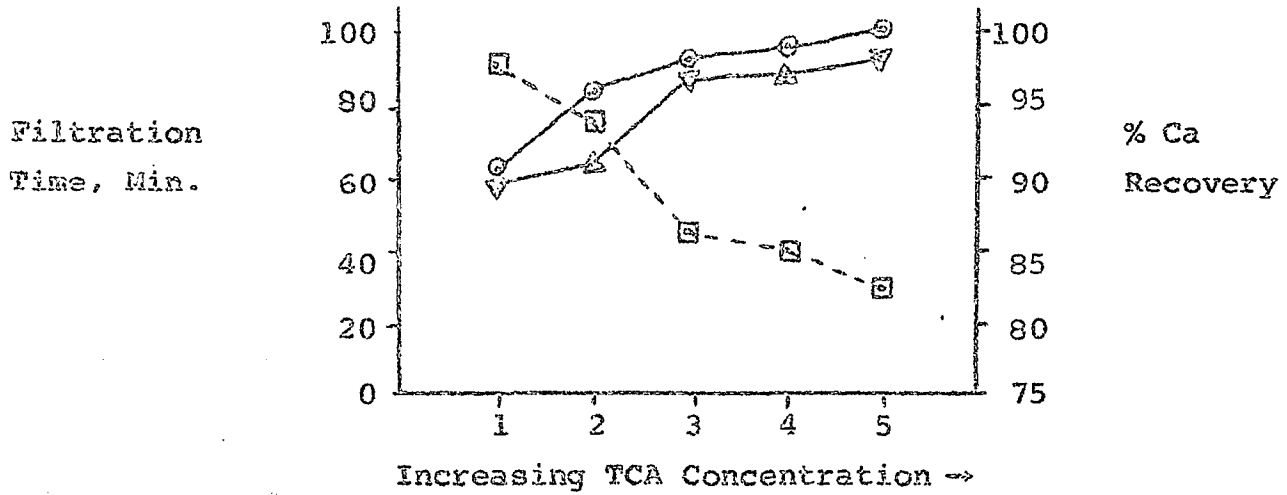
VI. pH curve for TCA.

A standard filtrate solution was prepared by precipitating a 1000 ml milk sample with 150 ml of 6 N HCl. The solution was then filtered and washed with approximately 50 ml 3 N HNO₃. After neutralizing the filtrate with NaOH, it was made up to 2000 ml volume.

The pH curve was made by taking a 200 ml aliquot of the neutral filtrate, adding 20 ml of 5 N KOH, and titrating with 50% TCA solution. A pH meter was used and the pH plotted against the corresponding volume of TCA used.

The TCA concentration of a 200 ml sample aliquot could then be determined by adding 20 ml of 5 N KOH, reading the pH on the meter, and finding the corresponding TCA concentration on the graph.

I. TCA CONCENTRATION



- Filtration Time in Minutes
- △ Calcium Recovery from Filtrate Digested in HNO₃
- Calcium Recovery from Untreated Filtrate Samples

PRECIPITATION AND FILTRATION

Sample	ml TCA Used	Filtrate Appearance	Addition of Excess TCA to Filtrate	Filtration and Washing Time, Min.
I-1	100	Cloudy	More Precipitate Formed	90
I-2	200	Slightly Cloudy	No Precipitate	75
I-3	300	Clear	No Precipitate	45
I-4	400	Clear	No Precipitate	40
I-5	500	Clear	No Precipitate	30

EDTA-Ca TITRATION - UNTREATED FILTRATE

Sample	ml EDTA Used	ml Ca Solution Used	Ca g/l	Percent Recovery
10 ml Blank	1.41	0.40	1.15	100
20 ml Blank	3.20	2.18	1.13	
I-1	3.12	2.68	1.04	91
I-2	3.10	2.12	1.09	96
I-3	2.96	1.19	1.12	98
I-4	2.86	0.80	1.13	99
I-5	2.88	0.80	1.14	100

EDTA-Ca TITRATION - FILTRATE DIGESTED IN HNO₃

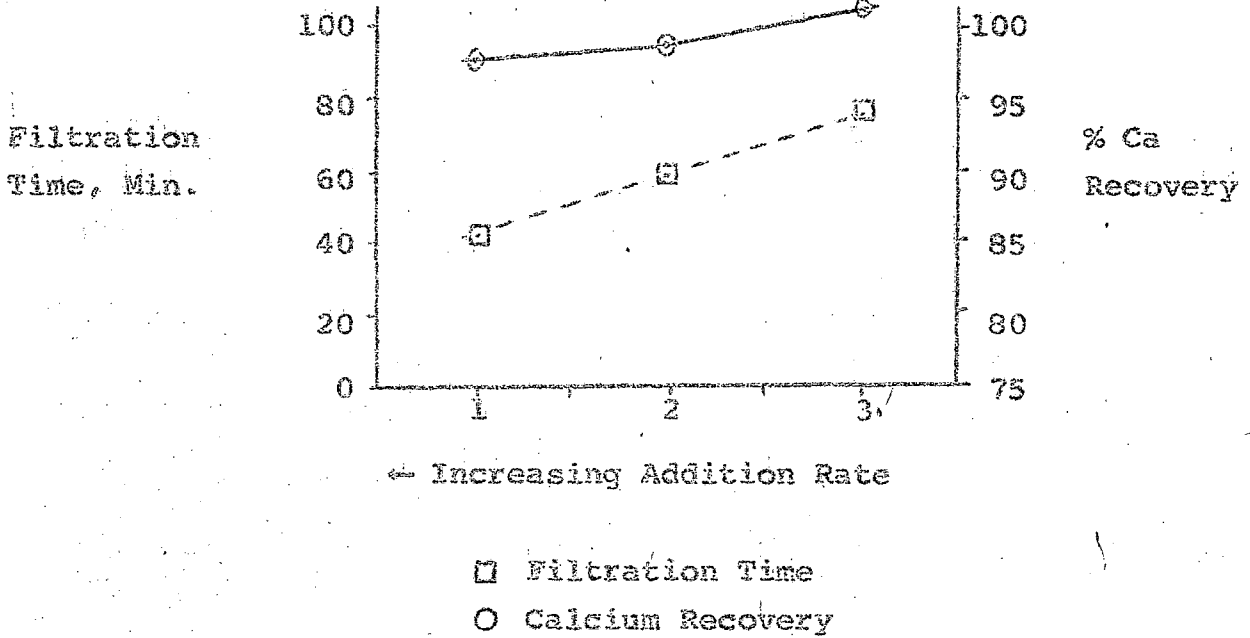
Sample	ml EDTA Used	ml Ca Solution Used	Ca g/l	Percent Recovery
10 ml Blank	1.40	0.40	1.15	101
10 ml Blank	1.40	0.48	1.14	100
I-1	2.61	0.81	1.02	90
I-2	2.75	1.34	1.03	91
I-3	3.02	1.72	1.10	97
I-4	3.35	3.02	1.10	97
I-5	2.90	1.04	1.12	98

Calculations

$$10 \text{ ml sample: } (\text{ml EDTA} \times 0.848) - (\text{ml Ca Solution} \times 0.1) = \text{Ca g/l}$$

$$20 \text{ ml sample: } (\text{ml EDTA} \times 0.424) - (\text{ml Ca Solution} \times 0.1) = \text{Ca g/l}$$

II. TCA ADDITION RATE



PRECIPITATION AND FILTRATION

Sample	II-1	II-2	II-3
TCA Addition	Rapid, Poured In At Once. 0 Min.	Poured Slowly-5 Min.	Added Dropwise-30 Min.
Precipitate Appearance	Lumpy	Medium Grain	Fine Grain
Filtrate Appearance	Clear	Clear	Slightly Cloudy
Filtration & Washing time	45 Min.	60 Min.	75 Min.
ml TCA Used	300	300	300

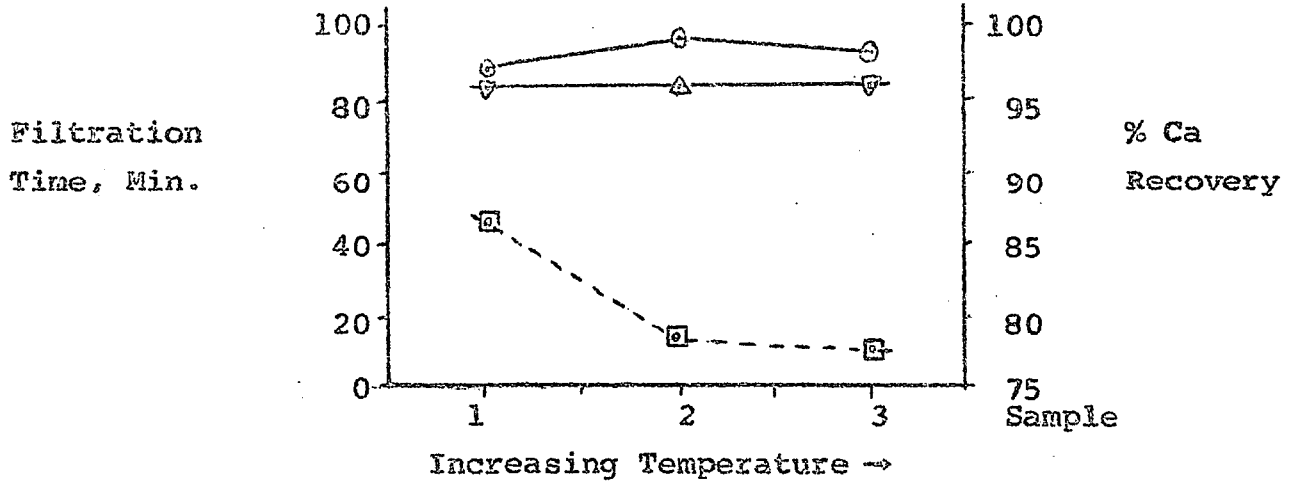
EDTA-Ca TITRATION

Sample	ml EDTA Used	ml Ca Solution Used	Ca g/l	Percent Recovery
Blank	1.58	1.13	1.21	100
Blank	1.55	1.32	1.20	99
II-1	1.54	1.03	1.18	98
II-1	1.52	0.86	1.18	98
II-2	1.50	0.67	1.18	98
II-2	1.52	0.75	1.19	99
II-3	1.55	0.73	1.22	101
II-3	1.55	0.73	1.22	101

Calculation

$$(\text{ml EDTA} \times 0.834) - (\text{ml Ca solution} \times 0.1) = \text{Ca g/l}$$

III. TCA - MILK TEMPERATURE



- Filtration Time, Minutes
- Calcium Recovery, EDTA Titration
- △ Calcium Recovery, Flame Analysis

PRECIPITATION AND FILTRATION

Sample	III-1	III-2	III-3
ml TCA Used	300	300	300
Temp. Milk TCA	20°C	40°C	80°C
Precipitate Appearance	Medium Grain	Very porous Granular	Very porous Granular
Precipitate Appearance	Clear	Clear	Clear
Filtration and Washing Time	45 Min.	15 Min.	12 Min.

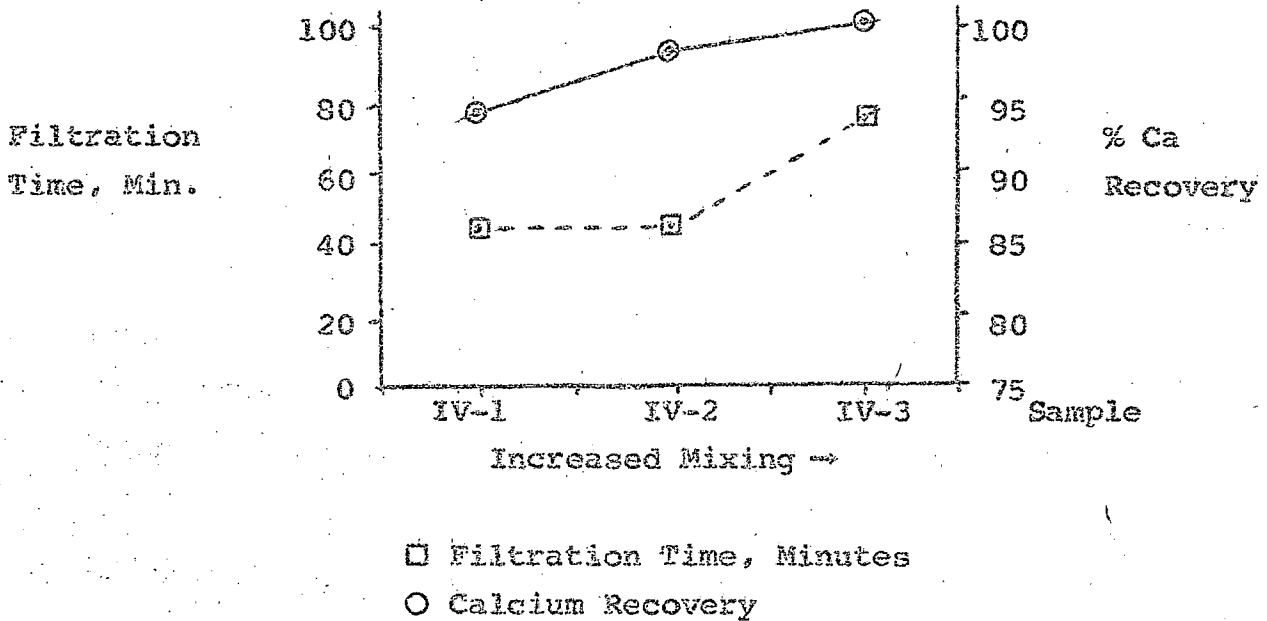
EDTA-Ca TITRATION AND FLAME ANALYSIS

Sample	ml EDTA Used	ml Ca Solution Used	Ca g/l	Percent Recovery
Blank	1.46	0.45	1.16	100
III-1	1.44	0.64	1.13	97
III-2	1.49	0.80	1.15	99
III-3	1.42	0.38	1.14	98
Blank	Flame Photometer Analysis		1.20	100
III-1	Flame Photometer Analysis		1.15	96
III-2	Flame Photometer Analysis		1.15	96
III-3	Flame Photometer Analysis		1.15	96

Calculation

$$(ml EDTA \times 0.828) - (ml Ca Solution \times 0.1) = Ca g/l$$

IV. MIXING OF MILK - TCA SOLUTION



PRECIPITATION AND FILTRATION

Sample	IV-1	IV-2	IV-3
ml TCA Used	300	300	300
Stirring	Slight Stirring Glass Rod	Vigorous Stirring Glass Rod	Mechanical Stirring 15 Min.
Precipitate Appearance	Gelatinous Fine Grain	Medium Grain	*
Filtrate Appearance	Cloudy	Clear	*
Filtration and Washing Time	45 Min.	45 Min.	*

*Insufficient milk to run this sample. Sample II-3 is shown on graph due to similarity of sample treatment.

EDTA-Ca TITRATION AND FLAME ANALYSIS

Sample	ml EDTA Used	ml Ca Solution Used	Ca g/l	Percent Recovery
Blank	1.46	0.45	1.16	100
IV-1	1.40	0.70	1.09	94
IV-2	1.44	0.64	1.13	98
Blank	Flame Analysis		1.20	100
IV-1	Flame Analysis		1.10	92
IV-2	Flame Analysis		1.15	96

Calculation

$$(\text{ml EDTA} \times 0.628) - (\text{ml Ca Solution} \times 0.1) = \text{Ca g/l}$$

V. FROZEN MILK

PRECIPITATION AND FILTRATION

Sample	ml TCA Used	Sample Appearance	Precipitate Appearance	Filtrate Appearance	Filtration & Washing Time
V-1	300	Partially Denatured	Dry, Porous	Clear	15 Min.

EDTA-Ca TITRATION AND FLAME ANALYSIS

Sample	ml EDTA Used	ml Ca Solution Used	Ca g/l	Percent Recovery
Blank	1.46	0.45	1.16	100
V-1	1.44	0.58	1.13	98
V-1	1.46	0.91	1.12	97
Blank	Flame Analysis		1.20	100
V-1	Flame Analysis		1.14	95

VI. pH - TCA CONCENTRATION

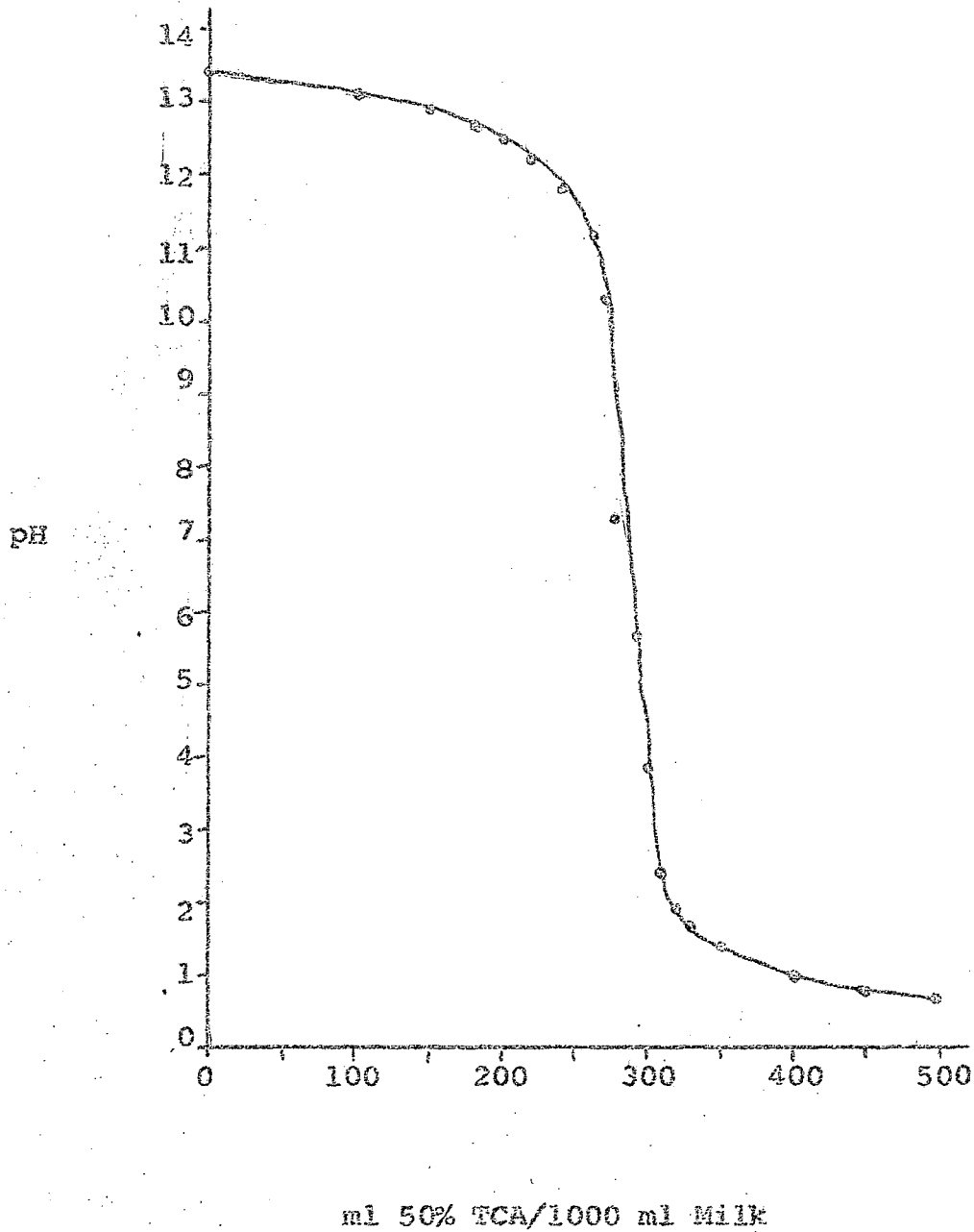
pH AND TCA CONCENTRATION OF STANDARD SOLUTIONS

TCA Conc. ml/l Milk	pH	TCA Conc. ml/l Milk	pH	TCA Conc. ml/l Milk	pH
0	13.4	270	10.3	330	1.7
100	13.1	280	7.3	350	1.4
150	12.9	290	5.7	400	1.0
200	12.5	300	3.9	450	0.8
240	11.8	310	2.4	500	0.7
260	11.2	320	1.9		

TCA CONCENTRATION IN MILK FILTRATE

Sample	ml Milk Used	ml TCA Added	pH of Filtrate	ml TCA Found	ml TCA Used
1	1000	100	13.3	50	50
2	1000	200	12.9	150	50
3	1000	300	11.3	258	42
4	1000	400	1.2	375	25
5	1000	500	0.8	450	50

VI. pH - TCA CONCENTRATION



DISCUSSION OF RESULTS

Considering the cost of reagents as well as the calcium recovery and filtering efficiency, the optimum trichloroacetic acid (TCA) concentration appears to be about 300 ml of 50% TCA per liter of milk. Increasing the TCA concentration by 60% (200 ml) over this amount only increases the calcium recovery by 2% and decreases filtering time by 15 minutes. Decreasing the TCA concentration by 60% lowers the calcium yield by as much as 8% and increases the filtering time by 45 minutes.

Slow addition of TCA solution to the milk increases the calcium recovery slightly (2%) but increases pouring and filtering time to 60 minutes.

Heating the milk and TCA adds little, (2%), to the calcium recovery but significantly reduces the time required for filtration. A simple method of pre-heating the samples might be worthy of further investigation.

The calcium recovery is somewhat dependent on the TCA-milk solution being mixed completely. Vigorous stirring with a glass rod is necessary for a good recovery.

Previously frozen milk filters much faster than a regular milk sample. This is due to the protein being partially denatured by the freezing process. The calcium recovery doesn't appear to be significantly altered by freezing.

The loss of approximately 50 ml of TCA across the precipitation-filtration step is probably due to occlusion in the precipitate but the possibility exists of a reaction occurring which would account for the loss.

CONCLUSION

The current method¹ meets nearly all of the requirements for a maximum calcium-strontium yield if the procedure is followed strictly. The one improvement which might be made is in pre-heating the milk to speed up the filtration step.

REFERENCES

(1) "Determination of Strontium-89 and Strontium-90 in Whole Milk. Nitric Acid Procedure." (August 14, 1962) p. 2, SWRHL, Las Vegas, Nevada.

(2) "A Rapid Determination of Calcium in Milk Using Ethylenediaminetetraacetate (Di-Sodium) as a Titrant." F. B. Johns, M. Knox, D. Moden, and E. Halker. Division of Radiological Health, U. S. Public Health Service (October 1962).

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