USING ATOMIC ABSORPTION SPECTROPHOTOMETRY TO MONITOR CALCIUM IN CLEANING SOLUTIONS FLOWING IN A MILK PIPELINE[1, 2]

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ABSTRACT

Concentrations of calcium in mixtures of acid and alkaline detergents containing milk were determined by atomic absorption spectrophotometry. Four concentrations of milk (0.0, 0.01, 0.1, and 1.0%) were injected into flowing water containing four concentrations of acid or alkaline detergent (0.0, 0.03, 0.3, and 3.0%) at each of three temperatures (20, 45, or 70 C). The concentration of alkaline detergent was not a significant variable. Less calcium was detected in solutions of acid detergent at 20 C than in solutions at 45 or 70 C. An increase in concentration of milk from 0.01 to 0.1% resulted in an approximate 10-fold increase in the amount of calcium detected with both alkaline and acid detergents. When the concentration of milk was increased from 0.1 to 1.0%, the same 10 fold increase in the amount of calcium was noted for the acid detergent. However, 0.1 to 1.0% increase in the concentration of milk resulted in only a 7-fold increase in the amount of calcium detected in the alkaline detergent.

Residue on a milk-contact surface of storage and processing equipment is removed at a varying rate during the cleaning cycle. This rate of removal has been studied at different time increments *(2)*. If the rate of removal of the residue could be monitored continuously, a more accurate analysis of a cleaning cycle could be made.

Anderson et al. *(1)* found that, with little interference from either individual chemical ingredients or from changes in the solution temperature, an atomic absorption spectrophotometer could be used to detect the amount of calcium in solutions that contained milk, ingredients of commercial detergents, and water.

The object of this study was to determine the ability of the atomic absorption spectrophotometer to detect and measure the amount of calcium (milk) injected into alkaline and acid detergent solutions flowing in a piping system. The long-range objective of this research was to develop instrumentation for monitoring the rate of removal of constituents of residue from milk-contact surfaces during cleaning.

MATERIALS AND EQUIPMENT

Materials

A dry alkaline detergent (Klenzade HC-41, recommended concentration 1.0 – 3.0%) and a liquid acid detergent (Klenzade AC-3, recommended concentration 0.1%) were used as cleaning agents. A standard stock solution of 2000 mg calcium/l was prepared by dissolving 4.9945 g of dried $CaCO_3$ (analytical grade) in 6 N HCl and diluting the solution with distilled water to one liter.

To measure the amount of calcium in milk, standard solutions containing 0, 5, 10, and 15 mg calcium/l were prepared by diluting the standard stock solution with distilled water. Each standard solution and distilled water containing milk were aspirated into the spectrophotometer. Instrument responses were recorded and compared to determine the amount of calcium in the milk. For the acid detergent study, standard solutions containing 0.0, 0.16, 1.6, and 16.0 mg calcium/l were prepared by diluting the standard stock solution with acid detergent-water (DW) solution. These solutions were aspirated into the spectrophotometer, and instrument responses were recorded before and after each test to confirm the amount of calcium injected. For the alkaline detergent study, no standard solutions were used because the cleaning solution from the pipeline was force-fed into the spectrophotometer, and no method was available for force-feeding a standard solution at the same rate as the solution was aspirated during a test.

Equipment

The simulated cleaned-in-place (CIP) system (Fig. 1) consisted of a variable speed sanitary pump (7½ hp motor) with controller, a 60-gal detergent-water-solution reservoir, a milk injector, a turbine flow meter with a read-out unit, 105 ft of 1½-inch stainless steel tubing, and three air-actuated, stainless steel valves.

The milk injection unit consisted of a solenoid-operated valve, several stainless steel fittings, and a 20-inch length of plastic tubing. The plastic tubing was marked to indicate the volume of milk-water (MW) solution at various depths of filling.

An atomic absorption spectrophotometer (Varian Techtron, Model 1000), equipped with a calcium hollow cathode

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[1]Contribution from the University of Missouri Experiment Station, Journal Series No. 6808.

[2]Trade names and names of commercial companies are used in this publication solely to provide specific information. Mention of a trade name or manufacturer does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture or an endorsement by the Department over other products not mentioned.

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TABLE 1. MEAN INSTRUMENT RESPONSE AND AMOUNTS OF CALCIUM FOR KNOWN CONCENTRATIONS OF MILK

	Alkaline		Acid	
Conc. of milk (%)	Calcium (mg/l)	Instrument response (mv)	Calcium (mg/l)	Instrument response (mv)
0.0	0.0	0.0	0.0	0.0
0.01	0.16	2.03	0.13	1.12
0.1	1.62	20.40	1.59	13.55
1.0	15.58	131.81	16.76	126.22

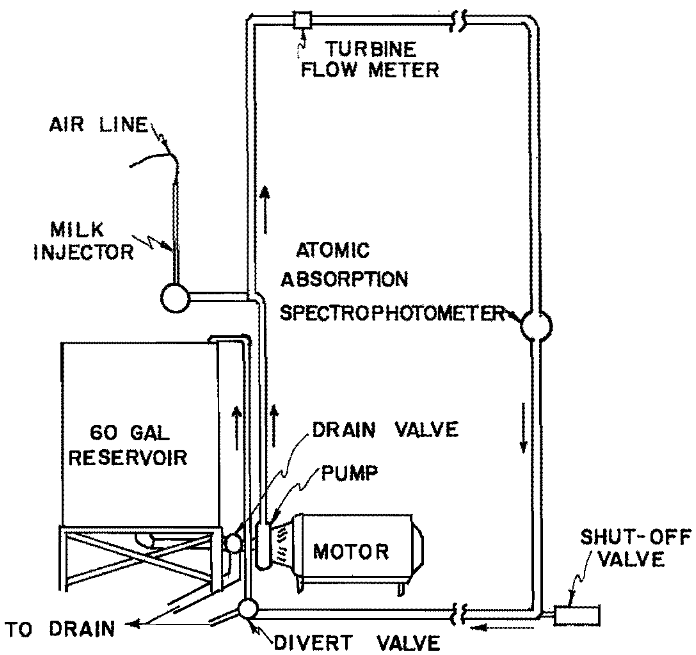

Figure 1. Schematic diagram of the simulated CIP system.

lamp, was used to measure the amount of calcium in solutions of milk-detergent-water (MDW). The dimensions of the nitrous-oxide burner were 6.324 cm × 0.457 mm. The slit width was 0.5 nm, and the lamp current was 6 ma. Acetylene was the fuel gas, and nitrous-oxide was the support gas. Signals from the spectrophotometer were fed into a two-pen, 10-inch chart laboratory recorder.

For the alkaline detergent study, a stainless steel needle and plastic tubing were used to force feed the MDW solution directly into the nebulizer by line pressure (Fig. 2). For the acid detergent study, a fluid control valve with assembly and plastic tubing were used to aspirate the MDW solution into the nebulizer (Fig. 3). The MDW solution was removed from the CIP return line via a 1/8-inch I.D. copper tube (directed upstream) attached to the fluid control valve (Fig. 3).

Procedure

The experimental design was a split-split plot with two replications. Each replication consisted of 12 series of four tests each. Four concentrations of milk (0.0, 0.01, 0.1, and 1.0%), four concentrations of detergent (0.0, 0.03, 0.3, and 3.0%), and three temperatures (20, 45, and 70 C) were used in all possible combinations.

The following procedure was used for each series of four tests: the reservoir was filled with softened water < 1 ppm calcium), and the required concentration (0.0, 0.03, 0.3, or 3.0%) of detergent was added. The DW solution was heated to the required temperature while it was circulated through the system at a velocity of approximately 5 fps.

Diluted milk samples (0.0, 0.01, 0.1, or 1.0%) were placed in the milk injector. When the DW solution reached the desired temperature, a test was started by injecting the milk into the DW solution at a constant rate for 20 sec. After the MDW solution passed through the test section, it was diverted to the drain to prevent contamination of the DW solution in the reservoir.

Figure 4 shows typical instrument responses (tracings) for four milk concentrations injected into solutions of alkaline and acid detergents during the study. After the 20-sec injection period, the tracing for each of the concentrations of milk returned to the baseline, a condition which indicated that the instrument had performed properly, and test conditions were valid.

To obtain an average peak response, data points obtained at 1-sec intervals for each injection curve (Fig. 4) were read into the computer. Quantities of calcium (mg/1) that caused these responses were determined by using standard curves. Variances in average peak values were compared by analyses of variance (ANOVA). Totals of the three-way interactions from the ANOVA program were used with orthogonal multipliers and with error A, B, and C terms to obtain sums of squares that were due to each of the 47 components *(6)*.

Those components that contributed significantly ($P < 0.05$) to the total sums of squares were used in a multiple regression computer program with mean values of the three-way interactions from the ANOVA program to obtain response surface equations for each detergent. Two equations, one linear and one polynomial, were required because the

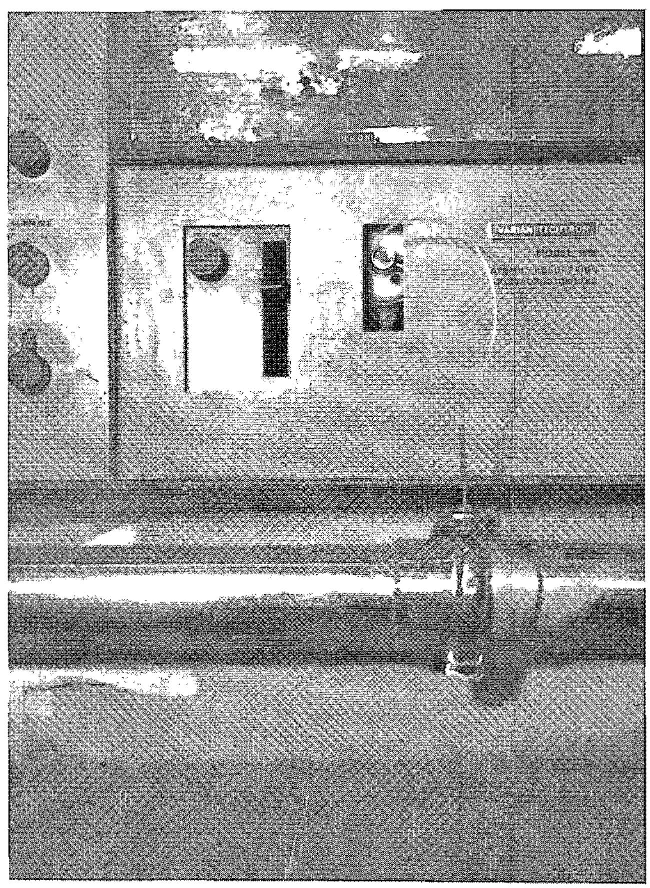

Figure 2. Stainless steel needle and plastic tubing used to force feed MDW solution into nebulizer.

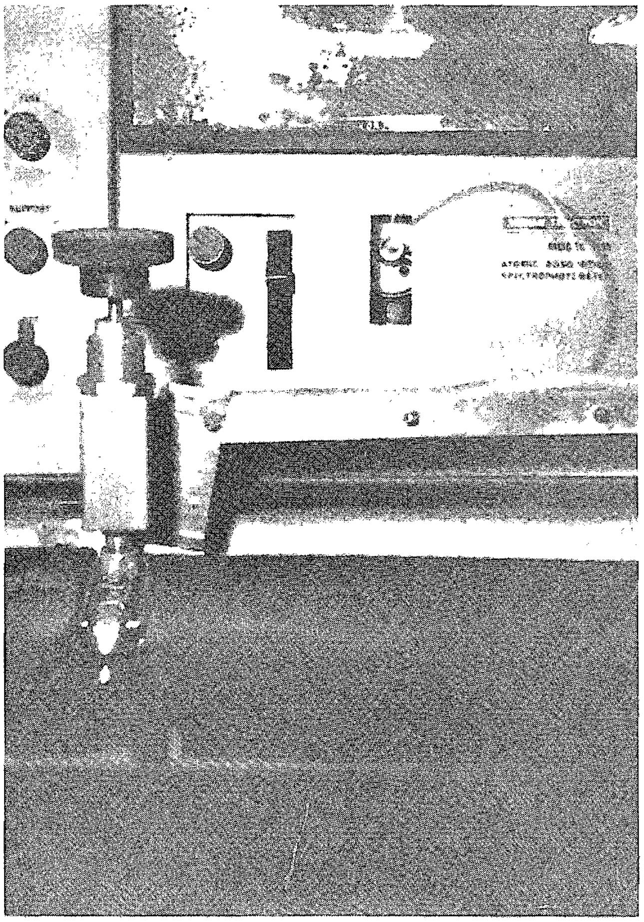

Figure 3. Fluid control valve with assembly and plastic tubing used to aspirate MDW solution into nebulizer.

polynomial equation became negative for very low concentrations of milk. These equations were used to obtain three-dimensional plots. Response surface equations for each detergent were used as standard curves to determine the total amounts of milk detected by the spectrophotometer. Instrument response from each test was analyzed using the standard curve for the detergent to predict the amount of milk injected. Means and standard deviations were determined for each variable.

RESULTS AND DISCUSSION

Effect of concentration of milk

Concentrations of milk tested were responsible for 95.4 and 97.1% of the experimental variations in the studies with alkaline and acid detergents, respectively. The mean instrument response (mv) and the amount of calcium (mg/l) for known concentrations of milk in alkaline and acid detergents are shown in Table 1. In the studies of both the acid and alkaline detergents, no significant differences ($P < 0.05$) were noted in the amount of calcium (mg/l) in solution with no milk and with 0.01% milk. The average sensitivity of the instrument to calcium (defined as a signal to noise ratio of 2:1) was 0.055 and 0.048 mg/l with solutions of 0.01% milk in alkaline and acid detergent solutions, respectively.

For the alkaline detergent studies, a 10-fold increase in the concentration of milk (from 0.01 to 0.1%) resulted in a 0.16 to 1.62 mg/l increase in the amount of calcium. This increase was equaled by a 10-fold increase (from 2.03 to 20.40 mv) in the response of the spectrophotometer. The next 10-fold increase in the concentration of milk (from 0.1 to 1.0%), which amounted to an increase in the amount of calcium from 1.62 to 15.58 mg/l, produced less than a 7-fold increase (from 20.4 to 131.8 mv) in the instrument response. The reduction in instrument response may have resulted from the increased viscosity of samples and the higher concentration of salts in the solution. Since salts crystallize in the nebulizer, instrument response is reduced *(4)*.

For the acid detergent studies, an increase in the concentration of milk from 0.01 to 0.1% amounted to a 12-fold increase (from 0.13 to 1.59 mg/l) in the amount of calcium and a 12-fold increase (from 1.12 to 13.55 mv) in the instrument response. However,

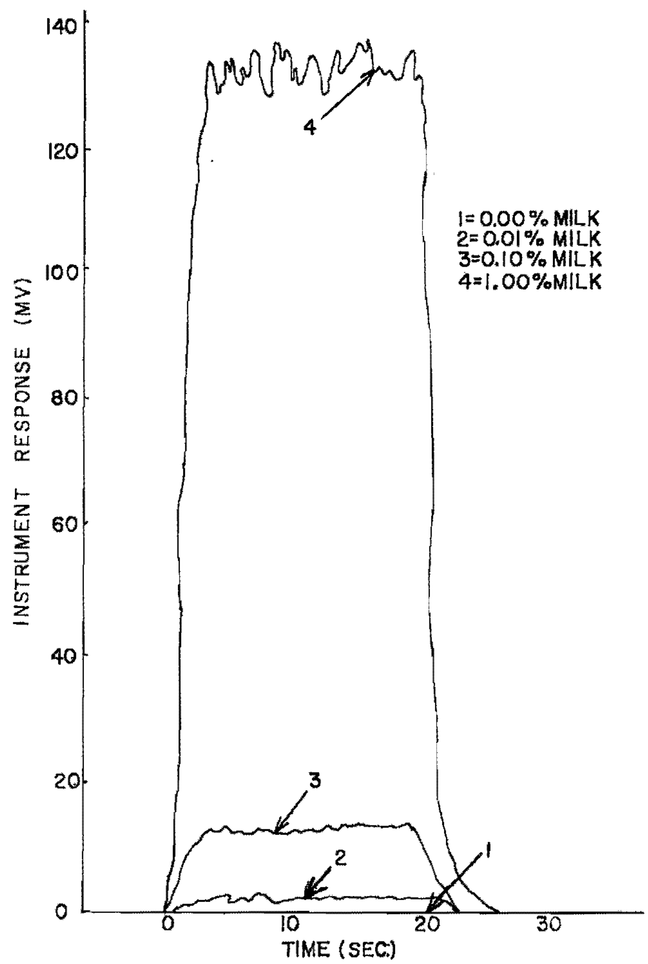

Figure 4. Typical instrument responses to four concentrations of milk injected into solutions of alkaline and acid detergents.

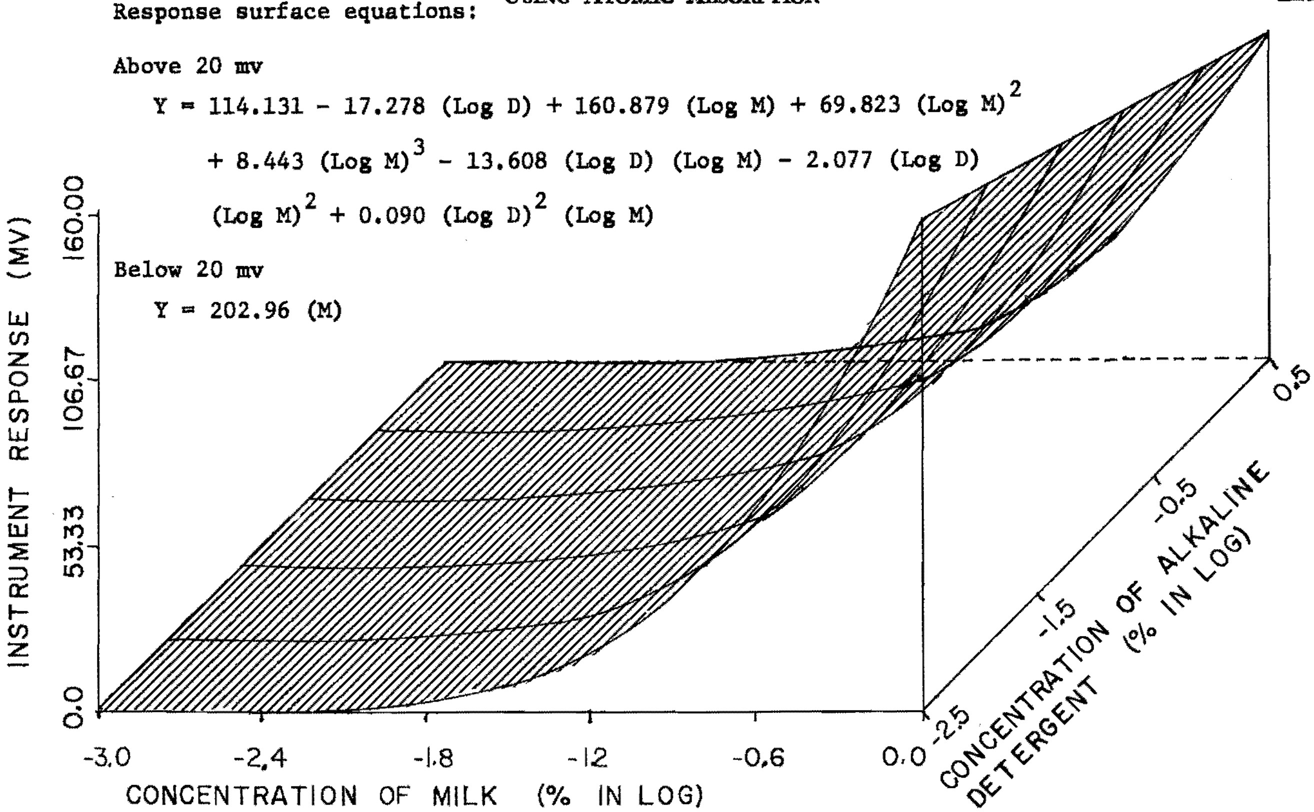

Figure 5. A plot of the response surface equation for instrument response (mv) as a function of concentration of milk (% in log) and concentration of alkaline detergent (% in log) using both straight line and polynomial equations.

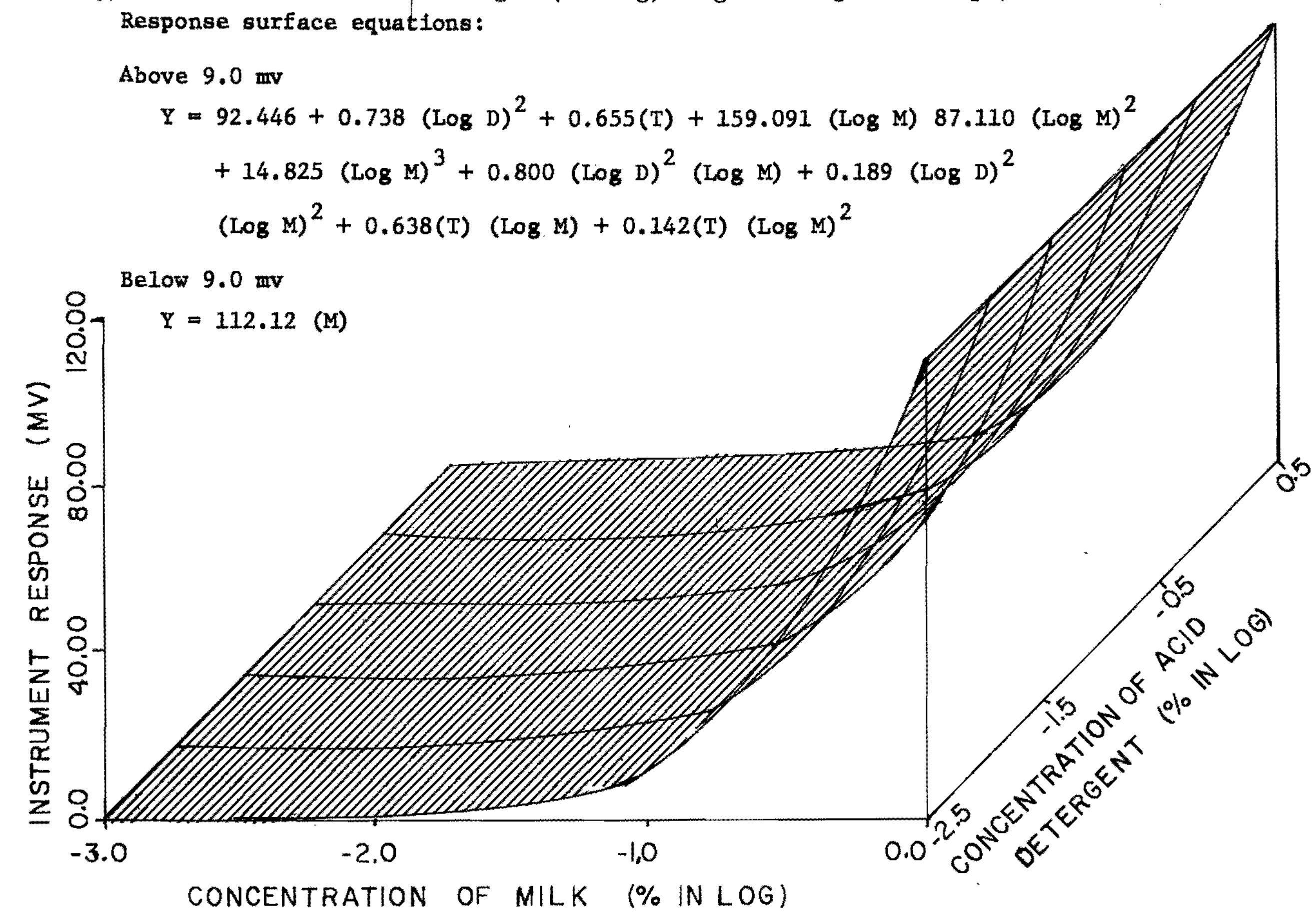

Figure 6. A plot of the response surface equation for instrument response (mV) as a function of concentration of milk (% in log) and concentration of acid detergent (% in log using both straight line and polynomial equations.

TABLE 2. OVERALL MEAN AMOUNTS OF MILK SOLIDS INJECTED AND MEASURED (MG), WITH THEIR STANDARD DEVIATIONS, FOR THREE CONCENTRATIONS OF MILK AND FOUR CONCENTRATIONS OF ALKALINE DETERGENT, AVERAGED FOR THREE TEMPERATURES

Concentration of milk (%)	Concentration of det. (%)	Amount measured (mg)		Amount injected (mg)	
		Mean	Standard deviation	Mean	Standard deviation
	0.0	384	34	390	69
	0.03	448	94	457	196
0.01	0.3	400	36	544	90
	3.0	401	23	271	63
		(408)[a]		(416)	
		4,180	591	2,620	421
	0.0	4,220	619	3,890	2,000
0.1	0.03	4,050	487	5,300	504
	0.3	4,240	484	4,420	1,970
	3.0	(4,170)		(4,060)	
	0.0	38,100	4,940	26,500	4,590
	0.03	41,900	9,190	42,900	10,400
1.0	0.3	42,600	4,240	50,000	13,000
	3.0	39,900	3,140	35,500	10,800
		(40,600)		(40,700)	

[a]Numbers in parentheses are the average amounts of milk solids for each concentration of milk.

TABLE 3. OVERALL MEAN AMOUNTS OF MILK SOLIDS INJECTED AND MEASURED (MG), WITH THEIR STANDARD DEVIATIONS, FOR THREE CONCENTRATIONS OF MILK AND FOUR CONCENTRATIONS OF ACID DETERGENT, AVERAGED FOR THREE TEMPERATURES

Concentration of milk (%)	Concentration of det. (%)	Amount injected (mg)		Amount measured (mg)	
		Mean	Standard deviation	Mean	Standard deviation
	0.0	402	69	415	101
	0.03	400	47	359	97
0.01	0.3	413	60	447	69
	3.0	424	43	389	72
		(409)[a]		(402)	
	0.0	3,650	610	3,610	617
0.1	0.03	4,690	859	3,750	849
	0.3	3,840	362	4,160	311
	3.0	4,170	487	4,670	391
		(4,090)		(4,050)	
	0.0	36,100	5,870	37,500	8,860
1.0	0.03	39,000	3,300	36,600	6,540
	0.3	39,500	4,180	38,400	5,100
	3.0	43,600	3,250	43,000	4,650
		(39,600)		(38,900)	

[a]Numbers in parentheses are the average amounts of milk solids for each concentration of milk.

an increase (from 0.1 to 1.0%) in the concentration of milk, which resulted in an increase in the amount of calcium from 1.59 to 16.76 mg/l, caused a 9.3-fold increase (from 13.5 to 126.22 mv) in the instrument response. The reduced instrument response was probably a result of the increased viscosity of the samples.

Effect of temperature

The temperature of the solution had no effect on the amount of calcium measured in the alkaline detergent. The fact that the alkaline detergent sample was force fed into the nebulizer, rather than aspirated, may have eliminated some of the effects of temperature. or the acid detergent studies, the amounts of calcium measured at 20 C were slightly but significantly lower than those measured at 45 or 70 C. This reduction in the amount of calcium measured may have been caused by an increase in the viscosity of the acid detergent samples at the lower temperature. Since the acid detergent samples were aspirated into the nebulizer, viscosity would have affected rates of aspiration.

Effect of detergent

Except at a 1.0% concentration of milk, no significant differences were noted in instrument response (mv) due to concentration of alkaline or acid detergent. For 1.0% milk, the instrument response decreased as the concentration of alkaline detergent increased, and significant detergent-milk interaction occurred. The instrument response was 154.23, 144.16, 134.71, and 95.00 mv for 0.0, 0.03, 0.3, and 3.0% concentrations of alkaline detergent, respectively. The decrease in instrument response may have resulted from formation of thermally stable compounds in the nitrous oxide flame.

The response surface equations and the plot of the equations for instrument response (mv) as a function of the concentrations of milk (% log) were used to obtain the response surfaces for the alkaline detergents (Fig. 5) and acid detergents (Fig. 6). These instrument response surfaces were used to predict the amount of milk injected.

Quantitation of milk injected–alkaline study

Overall mean amounts of milk solids injected and measured (mg) and their standard deviations for three concentrations of milk and for four concentrations of alkaline detergent are averaged for three temperatures (Table 2). For a 0.01% concentration of milk, the mean amount of milk solids injected for the alkaline detergent was 408 mg, and the mean amount measured was 416 mg. The standard deviation for the amount of milk solids measured was greater than the standard deviation for the amount injected. This might be expected because the variation that occurred during injection should have been included in the variation of the amount measured. For a 0.1% concentration of milk, the mean amount of milk solids injected was 4,170 mg, and the mean amount measured was 4,060 mg. The coefficient of variation for amount of milk injected for each concentration of alkaline detergent varied from 10 to 15%. The amount of milk solids measured for a 0.0% concentration of alkaline detergent was low, 2,620 mg. For a 1.0% concentration of milk, the mean amount of milk solids injected for the alkaline detergent was 40,600 mg; the mean amount measured was 40,700 mg. The coefficient of variation for amount of milk solids injected for each concentration of alkaline detergent ranged from 8 to 15% except for the 0.03% concentration of alkaline detergent which had 43% coefficient of variation. The amount of milk solids measured for a 0.0% concentration of alkaline detergent was low, 26,500 mg. Coefficients of variation for amount of milk solids measured for each concentration of alkaline detergent were all high and ranged from 17 to 30%.

Quantitation of milk injected–acid study

The overall mean amounts of milk solids injected and measured (mg) and their standard deviations for three concentrations of milk and for four concentrations of acid detergent are averaged for three temperatures (Table 3). For a 0.01% concentration of milk, the mean amount of milk solids injected for the acid detergent was 409 mg; the amount measured was 402 mg. The coefficient of variation for the amount of milk solids injected for each concentration of acid detergent ranged from 10 to 17%. The coefficient of variation for amount of milk solids measured for each concentration of acid detergent ranged from 15 to 27%.

For a 0.1% concentration of milk, 4,090 mg of milk solids were injected, and 4,050 mg of milk solids were measured. The coefficient of variation for the amount of milk solids injected ranged from 9 to 18%. The coefficient of variation for mean amounts of milk solids measured for each concentration of acid detergent ranged from 8 to 23%. When an average of 39,600 mg of milk solids was injected (1.0% milk), an average of 38,900 mg of milk solids was measured. The coefficient of variation for amount of milk solids injected ranged from 7 to 16%. The coefficient of variation for the amount measured ranged from 11 to 18%

The above discussion indicates that considerable variations occurred in these data. These variations were most likely a result of differences in instrument adjustment and instrument drift. A slight change in flame setting gave a substantial change in instrument response. In atomic absorption spectrophotometry, standard solutions are usually tested before and after an unknown solution is analyzed to reduce errors that are due to variations in settings. In these tests, it was impossible to obtain the same setting each day. These variations in settings do not, however, detract from the method's potential use for monitoring the presence of calcium in a MDW solution on a continuous basis.

CONCLUSIONS

The following conclusions were reached: *(a)* concentration of milk accounted for most of the variation in the data; *(b)* the atomic absorption spectrophotometer can be used to measure the amount of milk flowing in a piping system; *(c)* the two formulated commercial detergents, one acid and one alkaline, did not significantly interfere with measurement of calcium; *(d)* because of the method of analysis used, a significant difference in quantities of calcium in solutions with no milk and 0.01% milk was unable to be detected; and (e) temperature of the solution did not, for practical purposes, affect the amount of calcium measured.

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