J. Milk Food Technol. Vol. 38, No. 10, Pages 590-593 (October, 1975)

Measurement by Spectrophotometry of Concentrations of Raw Milk in Circulating Solutions of Detergents[1]

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(Received for publication March 14, 1975)

ABSTRACT

Studies were made with alkaline and acid detergent solutions at three temperatures (20, 45, and 70 C) and four milk concentrations (0.00, 0.01, 0.10, and 1.0%). Turbidity measurements were made continuously while the solutions were circulating through a piping system. The turbidity of milk-detergent-water solutions was found to be primarily influenced by the concentration of milk. As little as 0.01% milk caused a significant change in turbidity (1.4%), and the percentage of transmittance decreased an average of 78.6 when 1% milk was added to the water that contained the detergent. Detergent concentrations and temperature of the solutions had only minor effects on turbidity. Formation of precipitates by action of phosphates in hard water did not decrease the sensitivity of the method to added milk.

The cleaning cycle of a cleaned-in-place (CIP) dairy processing system includes four phases: rinse, alkali, acid, and postrinse. The CIP cleaning techniques now use arbitrary time limits on each phase of the cycle. Time, materials, and energy could be saved if each phase were terminated when cleaning is complete. Controls cannot be properly designed until some property of the milk-detergent-water (MDW) mixture is found that can be continuously measured or monitored under "on-line" operating conditions.

The white color of milk is caused by reflected light that is scattered by minute fat globules and colloidal calcium caseinate and calcium phosphate (*5*). These components of milk are normally found in cleaning solutions after use. We previously reported that ingredients normally used in the formulation of commercial detergents, with the exception of wetting agent, do not interfere with the turbidimetric measurement of milk in water that contains the individual ingredient (*3*).

In the present study a spectrophotometer was used to continuously monitor changes in the turbidity of MDW solutions flowing in a pipe. Relationships of turbidity to milk concentration in the MDW solution for both the alkaline and acid phases of cleaning were studied under "on-line" operating conditions.

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MATERIALS AND METHODS

Materials

Two commercial detergents, a dry alkali (Klenzade HC-41) and a liquid acid (Klenzade AC-3) were used in studies with the circulating system. The piping system and method of adding milk to the system have been reported (*1, 2*). Turbidity of solutions was determined in a

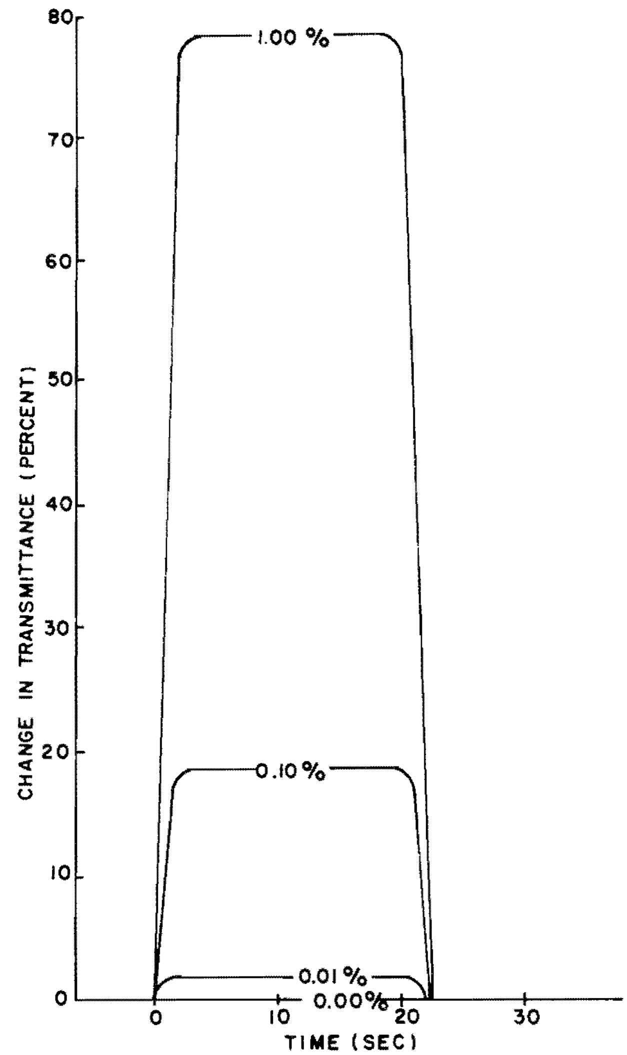

Figure 1. *Typical instrument responses for each of the four concentrations (%) of milk*

Bausch and Lomb Spectronic 20 equipped with a locally fabricated ½-inch-diameter flow-through cuvette.

Eighteen commercial detergents, sold for cleaning dairy equipment, were used to study effects of hard water (230 and 342 mg/l) on turbidity. Changes in turbidity caused by these detergents were measured in a standard cuvette.

Methods

The experimental design used with the circulating system was a split, split plot with two replicates. Each replicate consisted of 48 treatments. Four concentrations of detergent (0.0, 0.03, 0.3, and 3.0%), three temperatures (20, 45, and 70 C), and four concentrations of milk (0.0, 0.01, 0.1, and 1.0%) were studied in all possible combinations. The same experimental design was followed for both detergents.

Before each series of tests, the solution reservoir was filled with softened water (<5 ppm calcium), the required concentration (0.0, 0.03, 0.3, and 3.0%) of detergent was added, and the detergent-water (DW) solution was heated to the required temperature while being recirculated through the system at approximately 150 cm/sec.

A small portion of the solution flowing in the pipe was diverted continuously through a flow-through cuvette. The spectrophotometer was set at 527 nm, the wavelength of maximal absorbance of milk in water, and was adjusted to read 100% transmittance with the DW solution. Milk was then injected into the DW solution at a constant rate for 20 seconds. The instrument response was recorded on a strip chart recorder as change in the percentage of transmittance. Typical recordings for the four concentrations of milk are given in Figure 1.

The two sets of data analyzed for each experiment were: (*a*) average maximum change in instrument response and (*b*) total milligrams of milk solids measured as compared with the amount injected. Data points selected from the maximum response portion of each curve (flat portion) at 1-sec intervals were averaged to obtain the average maximum change in instrument response. Differences in average maximum changes produced by the same concentrations of milk were compared by analysis of variance (ANOVA). Totals of the three-way interactions from the ANOVA were used with the error mean square to obtain the sums of squares that were assignable to each of the 47 orthogonal components (5).

Response equations for each detergent were obtained by using, in a multiple regression computer program, those components that contributed significantly ($P > 0.05$) to the total sum of squares. Response equations for each detergent were used as standard curves to determine the total amount of milk detected by the spectrophotometer. The response equations and plots of the equations showing transmittance as a function of milk concentration in alkaline and acid detergents are given in Figures 2 and 3, respectively. Each measured value (change in percentage of transmittance) for each time interval (sec) was converted into its equivalent in milligrams of milk solids by using the response equations. The amounts of solids for each second were then summed over the total test time to obtain the total milligrams of solids measured.

A study was conducted to measure effects on turbidity of interactions of 18 commercial detergents with hard water (230 mg/l). Solutions were prepared in BOD bottles at concentrations recommended by the manufacturers. Milk was added at concentrations of 0.0, 0.01 and 0.1%. Samples were shaken, heated to 70 ± 2 C, and examined for turbidity. There were three replications.

In addition, tests were made to determine effects of time of holding on turbidity of solutions containing milk and 19 detergents. Milk (0.0 and 0.1%) was added to solutions containing manufacturer's recommended concentrations of detergent in hard water (342 mg/l). Hardness of the water was increased with $CaCl_2$ to provide a more informative test. Samples were shaken, heated to 70 ± 2 C and examined for turbidity. The samples were reheated daily for 8 h per day for 4 days. Turbidity measurements were taken daily.

RESULTS AND DISCUSSION

Effects of main variables-alkaline detergent

Data in Table 1 illustrate effects of the major variables for the experiments with alkaline detergent. The combined main effects of milk accounted for 99.7% of the total sum of squares attributable to regression. No significant differences ($P < 0.05$) were noted as a result of

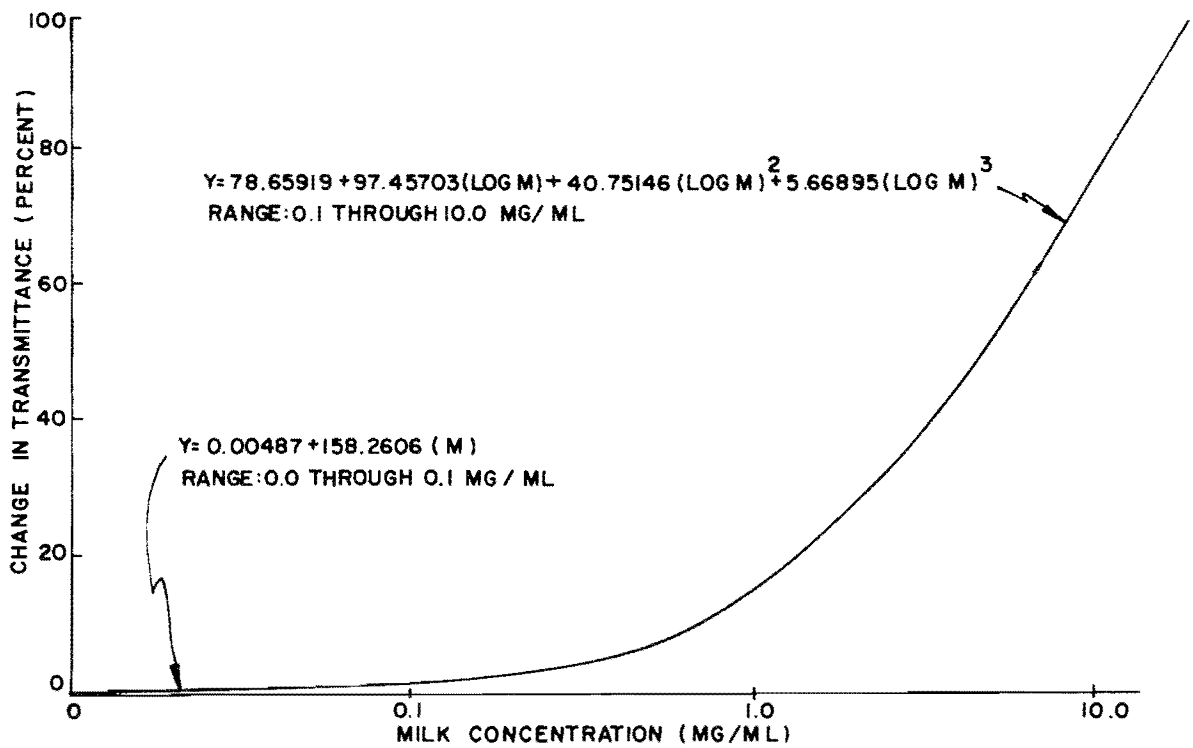

Figure 2. *Response equations and a plot of equations showing transmittance as a function of milk concentration in an alkaline detergent solution*

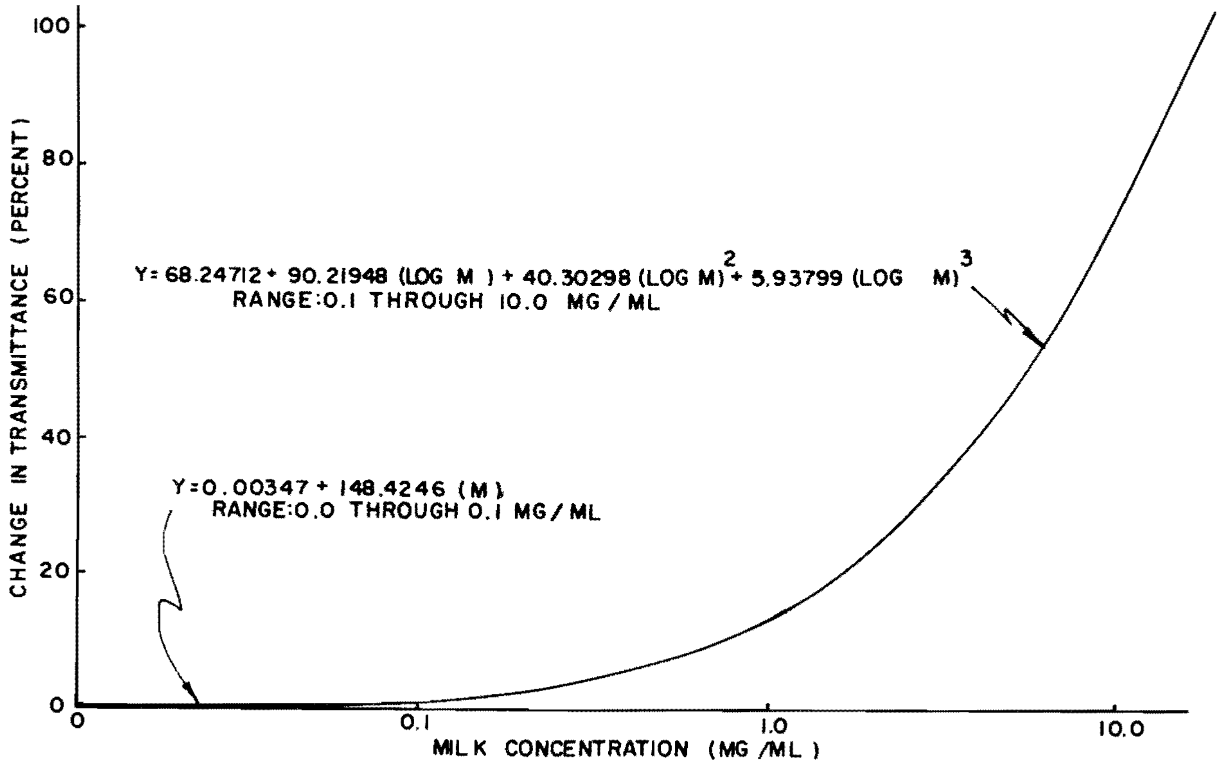

Figure 3. *Response equations and a plot of equations showing transmittance as a function of milk concentration in an acid detergent solution*

TABLE 1. *Average maximum change in percentage of transmission for four concentrations of alkaline detergent, three temperatures, and four concentrations of milk*

Alkaline detergent concentration, %	0.00	0.03	0.30	3.00
Mean, change in %T[a,b]	25.2	24.9	23.2	23.0
Temperature, C	20	45	70	
Mean, change in %T	24.1	24.0	24.2	
Milk concentration, %	0.00	0.01	0.10	1.00
Mean, change in %T	0.00	1.40	16.3	78.6

[a]% T = percentage of transmission.

[b]Underscored values indicate no significant difference at the 5.0% level.

changes in concentrations of detergent or temperature. Therefore, the effects of concentration of detergent and differences in temperature were not used in the development of the response equations (Figure 2). A concentration of 0.01% milk caused a mean change of 1.4% in the percentage of transmittance. Concentrations of 0.1 and 1.0% milk caused mean changes of 16.3 and 78.6%, respectively, in the percentage of transmittance.

Measurement of total solids injected-alkaline detergent

Quantities of milk injected into flowing alkaline solutions were measured with acceptable accuracy (Table 2). Mean quantities of milk solids (mg) injected and measured, differences between the two, and respective standard deviations for each concentration of milk are given in Table 2. The coefficient of variation for the amounts of milk solids measured decreased with increasing concentration; the values for each increasing concentration were 9.3, 6.5, and 6.3%, respectively.

Percentage errors of -3.08, -0.50, and 2.00% were noted for the 0.01, 0.1, and 1.0% concentrations of milk, respectively. Percentage of error = (amount injected - amount measured/amount injected) × 100. A minus sign before the number indicates that the measured value was lower than the amount injected. Average percentages of error for 0.0, 0.03, 0.3, and 3.0% alkaline detergent were 4.66, 1.17, –2.80, and –4.61%, respectively. For the three temperatures the measured values, on the average, were in error by 3.10, –0.39, and –3.39%, respectively, for 20, 45, and 70 C.

TABLE 2. *Mean of quantities of milk solids (mg) injected and measured, differences between the two, and respective standard deviations for the alkaline detergent*

Conc. of milk (%)	Amount injected (mg)		Amount measured (mg)		Difference (mg)	
	Mean	Std. dev.	Mean	Std. dev.	Mean[a]	Std. dev.
0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.01	409	43	394	37	15	28
0.10	4172	421	4104	268	68	395
1.00	40624	4160	40858	2600	–234	4167

[a]Amount injected minus amount measured.

Effects of main variables - acid detergent

Milk markedly affected transmittance measurements, being responsible for 99.9% of the total sum of squares. No significant differences in instrument response were noted as a result of changes in concentration of acid detergent or temperature. These two variables were not used in formulating the response equation (Figure 3).

Comparisons among means (average maximum change in the percentage of transmittance) assigned to main effects of the variables involved in the formulated acid detergent experiment are shown in Table. 3. Only milk was a significant variable.

TABLE 3. *Average maximum change in percentage of transmission for four concentrations of acid detergent, three temperatures, and four concentrations of milk*

Acid detergent concentration, %	0.00	0.03	0.30	3.00
Mean, change in %T[a,b]	20.4	21.1	20.1	20.5
Temperature, C	20	45	70	
Mean, change in %T	21.0	20.2	20.4	
Milk concentration, %	0.00	0.01	0.10	1.00
Mean, change in %T	0.00	1.50	12.4	68.2

[a]% T = percentage of transmission.
[b]Underscored values indicate no significant difference at the 5.0% level.

Prediction of total solids injected - acid detergent

Means of quantities of milk solids (mg) injected and measured, differences between the two, and respective standard deviations for each concentration of milk are in Table 4. The coefficients of variation for amount injected for each increasing concentration of milk were 7.3, 13.5, and 10.1%, respectively. Coefficients of variation for amounts measured were 9.1, 9.7, and 5.8%, respectively. The percentages of errors for all concentrations of milk were negative, indicating that measured values were higher than the actual values. The percentages of error were –3.31, –4.56, and –6.75%, respectively, for the 0.01, 0.1, and 1.0% concentrations of milk. The average errors for 0.0, 0.03, 0.3, and 3.0% acid detergent were –5.38, –2.41, –1.51, and –5.32%, respectively. Measured quantities of milk solids detected averaged over all other variables had errors of 0.46, –3.77, and –6.73% for temperatures of 20, 45, and 70 C, respectively.

TABLE 4. *Mean of quantities of milk solids (mg) injected and measured, differences between the two, and respective standard deviations for acid detergent*

Conc. of Milk (%)	Amount injected (mg)		Amount measured (mg)		Difference (mg)	
	Mean	Std. dev.	Mean	Std. dev.	Mean[a]	Std. dev.
0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.01	410	30	396	36	14	27
0.10	4084	550	3777	369	307	289
1.00	39529	4010	37453	2176	2076	3481

[a]Amount injected minus amount measured.

Effects of hard water

Precipitates which form when hard water is softened by sequestering action of phosphates could conceivably interfere with transmittance measurements. The present experiments showed, however, that this is not a significant problem. Transmittance values for solutions of detergents to which no milk was added were as follows: 12 exceeded 99%, four were greater than 98%, one was 97%, and one was 83%. Formation of precipitate did not decrease the sensitivity of the transmittance tests. Decreases in transmittance averaged over all detergents with 0.01 and 0.1% milk added were 1.4 ± 0.25 and 14.0 ± 0.53%, respectively. Solutions containing the detergent that produced the greatest turbidity in water were observed to decrease in transmittance by 2.1 and 13.8% when 0.01 and 0.1% milk were added, respectively.

Effects of time

Because detergent solutions may be used to clean several pieces of equipment over a period of time, the effect of time on transmission was studied. Additions of 0.1% milk to hard water (342 mg/l) caused an initial mean change in transmittance of –14.5 ± 7.;% Turbidity stabilized after 2 days so the average difference in transmittance (%T, detergent without milk *minus* %T, detergent with 0.1% milk) was –11.0 ± 7.0%. Decreases in transmittance over the first 48 h averaged 8.7 ± 7.3% and 5.1 ± 6.0% for solutions with 0.0 and 0.1% milk, respectively. Increases in transmittance with time were observed for 3 solutions containing 0.1% milk.

CONCLUSIONS

From this study it was concluded that:

1. Changes in transmittance were mainly a function of milk concentration in solutions containing milk, detergent, and water.
2. Variations in temperature and concentration of detergent were not important factors.
3. Water hardness interacted with time of holding solutions causing variations in transmittance, but errors could be eliminated by observing transmittance prior to beginning the cleaning operation.
4. The spectrophotometer has good potential for monitoring concentrations of milk in solutions of detergents in CIP systems.

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