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Spectrophotometric Determination of Concentrations of Raw Milk in Solutions Containing Ingredients of Detergents[1]

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ABSTRACT

This paper reports effects of concentration of milk, concentration of individual ingredients of detergents, and temperature on transmittance of light at 527 nm through solutions of milk, water, and individual detergent ingredients. Milk in soft water caused the major portion of the variation, more than 99% of the total sum of squares, for each ingredient except sodium hydroxide and wetting agent. Milk contributed 95.7 and 17.9% to the sums of squares for the sodium hydroxide and wetting agent, respectively. Concentration of detergent ingredient and temperature were of practical significance only with wetting agent. Effects of milk on transmittance were significantly different among milk concentrations for *all* detergent ingredients, except trisodium phosphate in hard water at the lowest concentrations of milk.

Although precipitates contributed to turbidity of solutions containing trisodium phosphate in hard water, milk was responsible for 95.9% of the variance in the sum of squares. Additionally, in hard water milk accounted for 98.9, 99.1 and 99.7% of the sums of squares in analyses of effects on turbidity of sodium tripolyphosphate, sodium hexametaphosphate, and tetrasodium pyrophosphate, respectively. Turbidity increased with time of holding some phosphate solutions, however, this did not appear to pose serious problems in measuring content of milk.

The objective of this research was to determine the extent that chemical ingredients used in the formulation of commercial detergents interfere with spectrophotometric measurement of milk in aqueous solutions. The research is the initial step in investigating the possible use of the spectrophotometer as a device to measure quantities of milk residue in cleaning solutions as they pass through components of a milk processing plant or farm milking equipment.

The white color of milk is caused by the scattering of reflected light by minute fat globules, colloidal calcium caseinate, and calcium phosphate (*5*). Ashworth (*1*) observed that the turbidity per unit of concentration of total solids in milk varied with the fat content of the milk and with the efficiency of homogenization. About 75% of the total turbidity was attributed to the fat phase. Therefore, milk components in cleaning solutions, especially fat and protein, should be detectable by transmittance spectrophotometry, provided chemicals in the cleaning solutions do not interfere.

MATERIALS AND METHODS

Materials

Detergent ingredients, supplied by Economics Laboratory, Inc., St. Paul, Minnesota, consisted of four basic alkalies, three complex phosphates, two chelating agents, one wetting agent, one organic acid, and one mineral acid. Raw milk for the study was obtained from dairy farms at the University of Missouri-Columbia. Concentrations of fat and total solids in milk were determined by the Babcock test and the Mojonnier gravimetric method, respectively (*4*). Nonfat solids were determined by difference. Soft water ($<$ 2 ppm of calcium) used in the milk-ingredient-water (MIW) solutions was softened by ion exchange. Unsoftened water contained approximately 226 ppm of hardness. Three water baths were used to temper test solutions to 20, 45, or 70 ± 2 C.

A light spectrophotometer (Bausch and Lomb, Spectronic 20), set at a wavelength of 527 nm (wavelength of maximum absorbence obtained by scanning milk-water solutions), was used in analyses of the MIW solutions. Output signals were recorded with a dual-channel strip chart recorder.

Methods

The primary experiment was a 3 × 4 × 4 factorial in a randomized complete block with two replications. There were three temperatures (20, 45 and 70 C), four concentrations of detergent (they varied for each detergent ingredient), and four concentrations of raw milk (0.0, 0.01, 0.1, and 1.0%).

The same procedure was used to test each of the 12 ingredients. Various concentrations of the detergent ingredients were prepared in 300-ml BOD bottles. Ingredient concentrations were selected to cover ranges that would be used in the formulation of commercial detergents. Raw milk was added to each bottle to give the required milk concentration, and softened water was used to dilute the mixture to 300 ml.

In a second experiment each type of phosphate was prepared in hard water. The BOD bottles were shaken and placed in the appropriate water bath. When the solutions reached 20, 45 or 70 C, transmittance measurements were made. In addition, two concentrations each of four phosphates containing 0, 0.01, 0.1, or 1.0% milk were heated to and held at 70 C for 8 h per day during four days of storage. Transmittance measurements were made initially and at 24-h intervals.

Data were subjected to analyses of variance. Duncan's New Multiple Range Test (*3*) was used to determine significance of differences among means for each variable.

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RESULTS AND DISCUSSION

Effects of ingredients

Mean transmittance values (%) for four concentrations of each ingredient (indicated in parentheses) averaged over four concentrations of milk and three temperatures are shown in Table 1. Each detergent ingredient, except sodium hydroxide and the wetting agent, accounted for less than 1% of the total sum of squares.

Wetting agents lower interfacial tension between fat and water. Thus, they permit fat globules to break into smaller globules. The resulting large increase in total surface area would cause increased light absorption in the cuvette. We suggest, therefore, that increases in surface area caused the decreased transmittance we observed with increased concentrations of wetting agent ($P < 0.05$). Of the total sum of squares, 34.7% was attributable to the presence of the wetting agent. Although transmittance of the solution containing 0.3% sodium metasilicate was significantly higher ($P < 0.05$) than were those of solutions containing the other concentrations of this ingredient, the combined mean effect of this compound accounted for only 0.02% of the sum of squares. Sodium metasilicate is a relatively weaker alkali than is sodium hydroxide, and, therefore, had less effect upon turbidity. No significant differences

TABLE 1. *Mean transmittance values for four concentrations of detergent ingredients averaged over four concentrations of milk and three temperatures*

Ingredient and concentrations (%)[a]	Concentration of detergent			
	A	B	C	D
	Transmittance values (%)[bc]			
Sodium hydroxide (0.0, 0.45, 0.9, 1.35)	79.4	83.4	85.8	86.3
Sodium metasilicate anhydrous (0.0, 0.3, 0.6, 0.9)	76.6	77.9	77.3	77.0
Sodium carbonate (0.0, 0.3, 0.6, 0.9)	77.8	78.6	78.8	78.9
Trisodium phosphate (0.0, 0.75, 1.50, 2.25)	77.8	79.2	79.7	78.9
(Trisodium phosphate + hard water)	79.1	74.7	75.0	72.9
Sodium hexametaphosphate (0.0, 0.3, 0.6, 0.9)	75.8	76.7	76.8	76.6
(Sodium hexametaphosphate + hard water)	75.9	78.3	77.7	77.7
Sodium tripolyphosphate (0.0, 0.3, 0.6, 0.9)	78.2	79.5	79.0	78.2
(Sodium tripolyphosphate + hard water)	76.4	78.1	78.4	78.1
Tetrasodium pyrophosphate (0.0, 0.3, 0.6, 0.9)	74.5	77.1	77.0	76.6
(Tetrasodium pyrophosphate + hard water)	74.6	76.7	76.6	76.1
EDTA[d] plus sodium hydroxide (0.0, 0.21, 0.42, 0.63)	77.6	79.5	79.6	79.6
Sodium gluconate (0.0, 0.21, 0.42, 0.63)	77.1	77.9	78.3	78.6
Wetting agent DC-161 (0.0, 0.06, 0.12, 0.18)	78.6	38.0	29.4	26.1
Gluconic acid, 50% (0.0, 0.6, 1.2, 1.8)	77.5	78.6	79.2	79.1
Phosphoric acid, 75% (0.0, 0.6, 1.2, 1.8)	77.8	78.6	79.3	79.1

[a]Numbers in parentheses are concentrations of individual ingredients and correspond in order presented with transmittance values in columns A, B, C, and D.

[b]Each entry is an average of 24 readings; 12 are from each replication.

[c]Underscored values were not significantly different at the 5.0% level of probability according to Duncan's New Multiple Range Test.

[d]Ethylenediaminetetraacetic acid.

TABLE 2. *Mean transmittance values for four concentrations of milk averaged over four concentrations of detergent ingredient and three temperatures*

Detergent ingredient	Concentration of milk (%)			
	0.0	0.01	0.1	1.0
	Transmittance values (%)[a,b]			
Sodium hydroxide	99.5	98.5	91.6	45.3
Sodium metasilicate	99.6	98.0	86.1	25.0
Sodium carbonate	99.7	98.5	88.2	27.6
Trisodium phosphate	99.5	98.2	88.5	29.5
(Trisodium phosphate + hard water)	93.3	92.4	84.5	31.5
Sodium hexametaphosphate	98.3	96.8	85.8	25.0
(Sodium hexametaphosphate + hard water)	99.6	98.4	85.3	26.0
Sodium tripolyphosphate	99.6	97.6	86.6	31.0
(Sodium tripolyphosphate + hard water)	99.0	97.6	85.0	29.5
Tetrasodium pyrophosphate	98.8	96.8	85.6	24.0
(Tetrasodium pyrophosphate + hard water)	99.5	98.1	84.5	22.1
EDTA plus sodium hydroxide	99.3	98.2	89.2	29.5
Sodium gluconate	99.8	98.0	87.6	26.4
Wetting agent	56.0	52.6	46.0	17.4
Gluconic acid	99.3	98.1	88.2	28.7
Phosphoric acid	99.4	98.3	88.8	28.4

[a]Each entry is an average of 24 readings; 12 are from each replication.

[b]Underscored values indicate no significant difference at 5.0% level of probability according to Duncan's New Multiple Range Test.

were found among the concentrations of sodium tripolyphosphate, sodium hexametaphosphate, and gluconic acid.

Because trisodium phosphate softens by precipitation (2), it was expected that transmittance values would be lower (the precipitate would deflect the light) when it was dissolved in hard water rather than soft water. Tests were conducted with hard water and each of the four phosphates. Effects of adding the phosphates were significant, but effects of concentration were not significant except in the case of the highest concentration of tetrasodium pyrophosphate (Table 1). Transmittance decreased when trisodium phosphate were added to solutions of milk and water, but transmittance increased in solutions of the other three phosphates. Precipitate was visible in solutions containing trisodium phosphate, but the other solutions were clear.

Effects of milk

Mean transmittance values (%) for four concentrations of milk averaged over four concentrations of detergent ingredient and three temperatures are shown in Table 2. For each increasing concentration of milk, the mean transmittance value decreased significantly except for the comparison of 0.0 to 0.01% milk in the solution of trisodium phosphate in hard water.

Solutions of trisodium phosphate in hard water allowed considerably lower transmittance than solutions of this ingredient in soft water, 93.3 vs 99.5 percent. This obviously resulted from precipitation of calcium and/or magnesium phosphates. However, concentration of milk in hard water was responsible for 95.9, 98.9, 99.1, and 99.7 percent of the sums of squares in the analyses of variance for trisodium phosphate, sodium tripolyphosphate, sodium hexametaphosphate, and tetrasodium pyrophosphate, respectively.

In solutions made from soft water, milk accounted for more than 99.0% of the total sums of squares, except when sodium hydroxide and the wetting agent were present. Milk accounted for 95.7% of the variation in data when the ingredient was sodium hydroxide and for only 17.9% when the ingredient was the wetting agent. When data related to wetting agent were omitted, mean transmittance readings ranged from a high of 99.8% to a low of 98.3% when no milk was present; a high of 98.5% to a low of 96.8% for 0.01% milk; a high of 91.6% to a low of 85.6% for 0.1% milk; and a high of 45.3% to a low of 24.0% for 1.0% milk. For milk concentrations of 0.1 and 1.0%, sodium hydroxide gave the highest transmittance readings, probably because of its solubilizing effect on colloidal calcium phosphate and calcium caseinate.

The reduction of transmittance by wetting agent was readily apparent, as milk was responsible for only 18.2% of the total sum of squares. However, even in the presence of wetting agent all mean readings averaged over all the other variables for each concentration of milk were significantly different ($P < 0.05$).

Effects of temperature

Mean transmittance values (%) for three temperatures averaged over four concentrations of milk and four concentrations of detergent ingredient are shown in Table 3. No significant differences were noted as a result of changes in temperature for the following ingredients in soft water: sodium hydroxide, sodium metasilicate, trisodium phosphate, sodium tripolyphosphate, and sodium gluconate. For the other ingredients, an increase in temperature caused a significant ($P < 0.05$) decrease in transmittance, except for wetting agent. In this instance the opposite effect was noted. Although effects of temperature on certain ingredients were significant, they accounted for less than 0.1% of the total sums of squares, and the results are not discussed. The effect of

TABLE 3. *Mean transmittance values for three temperatures with four concentrations of milk and four concentrations of detergent*

Detergent ingredient	Temperature (C) 20	45	70
	Transmittance values (%)[a,b]		
Sodium hydroxide	83.7	84.1	83.3
Sodium metasilicate	76.9	77.3	77.3
Sodium carbonate	77.5	78.8	79.2
Trisodium phosphate	78.4	79.4	79.0
(Trisodium phosphate + hard water)	75.1	77.1	74.0
Sodium hexametaphosphate	75.7	76.7	77.1
(Sodium hexametaphosphate + hard water)	76.1	78.7	77.4
Sodium tripolyphosphate	78.9	78.6	78.6
(Sodium tripolyphosphate + hard water)	76.9	78.4	78.4
Tetrasodium pyrophosphate	75.2	76.4	77.2
(Tetrasodium pyrophosphate + hard water)	75.9	76.0	76.0
EDTA plus sodium hydroxide	78.5	79.3	79.4
Sodium gluconate	77.9	77.9	78.1
Wetting agent	68.4	32.8	27.9
Gluconic acid	77.4	77.9	80.5
Phosphoric acid	77.8	79.2	79.2

[a]Each entry is an average of 32 readings; 16 are from each replication.

[b]Underscored values indicate no significant defference at 5.0% level of probability according to Duncan's New Multiple Range Test.

temperature in the experiment involving the wetting agent was more pronounced and explained 25.7% of the total variation. The wetting agent used in the study seemed to markedly emulsify the milk fat at the higher temperatures.

In hard water, solutions containing trisodium phosphate or sodium hexametaphosphate were less turbid at 45 C than at either 20 or 70 C. Solutions of sodium tripolyphosphate were less turbid at 45 C and 70 C than at 20 C, but no difference was observed among solutions at 45 and 70 C. Temperature had no effect on transmittance of solutions of tetrasodium pyrophosphate.

Effects of time

Because polyphosphates in solution tend to revert to simple phosphates with time (*2*), effects of holding four types of phosphate solutions at 70 C for 8 h each day for 4 days were determined. Samples contained 0, 0.01, 0.1 and 1.0% milk. No changes in transmittance with time were observed with (*a*) the lower concentration (0.75%) of trisodium phosphate, (*b*) either concentration (0.3 and 0.9%) of sodium tripolyphosphate, or (*c*) the higher concentration (0.9%) of both sodium hexametaphosphate and tetrasodium pyrophosphate. Generally, transmittance decreased with time at the higher concentration (2.25%) of trisodium phosphate and the lower concentration (0.3%) of sodium hexametaphosphate and tetrasodium pyrophosphate (Table 4). Concentration of milk appeared to effect amounts of precipitation in solutions of only sodium hexametaphosphate (0.3%) in which there was no effect of time on transmittance in water or 0.01% milk.

TABLE 4. *Effects of time on percent transmittance of solutions of phosphates and milk in hard water*[a]

Phosphate	Days of storage	Percent milk			
		0.0	0.01	0.1	1.0
		Percent transmittance			
Trisodium (2.25%)	1	89.00	89.00	88.00	22.00
	2	90.00	88.50	75.50	21.00
	3	77.00	73.75	63.50	19.40
	4	61.75	59.50	52.75	21.75
Sodium hexameta (0.3%)	1	98.75	97.50	85.00	23.25
	2	99.75	97.00	85.50	24.00
	3	99.50	96.75	84.50	22.75
	4	97.38	96.25	77.75	20.00
Tetrasodium pyro (0.3%)	1	96.00	94.00	81.50	23.00
	2	94.00	91.75	80.50	22.50
	3	92.00	87.75	77.25	23.00
	4	82.00	78.75	68.00	19.50

[a]Results are shown for only those ingredients and concentrations for which there was an effect of time of storage.

Although transmittance increased with time under certain conditions, effects on determining concentration of milk by transmittance measurement were minimal. Results further suggested that it would not be difficult to formulate detergents in which time would have no effect on transmittance.

CONCLUSIONS

Major ingredients used in the formulation of commercial detergents, with the exception of the wetting agent, did not interfere with transmittance in milk-ingredient-water solutions to any practical extent. The wetting agent severly reduced transmittance. Except for the lowest concentration of milk, 0.01%, in solutions of trisodium phosphate in hard water, transmittance was lowered significantly by each stepwise increase in concentration of milk from 0.0 to 1.0%. Therefore, it appears that the light spectrophotometer may well be used to measure milk residue in cleaning solutions free from large concentrations of wetting agents like DC-161.

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