DETERGENCY OF CHLORINATED TRISODIUM PHOSPHATE ON MILK-PROTEIN SOILS FROM STAINLESS STEEL AND GLASS

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Detergency of Chlorinated Trisodium Phosphate on Milk Protein Soils from Stainless Steel and Glass

presented by

Everett Perkins Merrill

has been accepted towards fulfillment of the requirements for

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Major professor

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DETERGENCY OF CHLORINATED TRISODIUM PHOSPHATE ON MILK-PROTEIN SOILS FROM STAINLESS STEEL AND GLASS

By

Everett P. Merrill

AN ABSTRACT OF A THESIS

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science

ABSTRACT

DETERGENCY OF CHLORINATED TRISODIUM PHOSPHATE ON MILK-PROTEIN SOILS FROM STAINLESS STEEL AND GLASS

by Everett P. Merrill

Chlorinated alkaline detergents are used widely in the food industry for cleaning of stainless steel equipment where the benefit is manifested in brighter appearance and absence of spotting after cleaning and drying. Data on the cleansing function of chlorinated alkaline detergents were scarce and inconclusive when the present study was initiated. Recent postulations have been in general agreement with the data of this study, showing that chlorinated alkaline detergents increase protein solubility. This investigation of chlorinated alkaline detergency is believed significant for the methods of study used, as well as for the results obtained.

Measurements of skimmilk film deposited on glass and stainless steel surfaces were made. A gravimetric method was found to be superior to an interferometer, an ellipsometer, light transmission, and an electron microscope procedure for this purpose. When glass slides were dipped into skimmilk, the gravimetric method showed that after the first dip, the weight of film formed on the surface of the slide was a linear function of the number of times the slides were dipped.

Everett P. Merrill

The electron micrographs revealed marked unevenness of skimmilk films and showed why wetting ability and penetrability were desirable in removing milk-film soils.

Tenacious milk-protein films were made by dipping micro cover glass slides successively and momentarily into suspensions of clarifier slime and into solutions of calcium chloride, trisodium phosphate, and sodium hypochlorite.

Quantitative measurements of cleaning were made by a combination gravimetric-microkjeldahl method. This method was very effective in measuring residual films after a detergent treatment and was useful for making qualitative observations of cleaning during the washing procedure.

Chlorination of trisodium phosphate solutions, by addition of sodium hypochlorite, significantly increased the solubility of protein films on glass and stainless steel when the surfaces were washed for 10 minutes with the solution at a temperature of 65° C. and a pH of 11.5 \pm 0.1. The beneficial effect was obtained, using either distilled or hard water. The hard water contained 340 p.p.m. of total hardness. The trisodium phosphate solutions did not show a beneficial effect from chlorination when held at less than 65° C., and pH 11.5 \pm 0.1. The effect of chlorination in solubilizing protein was not attained until after one minute of exposure, but advanced rapidly thereafter with increasing time. A build-up, rather than removal, of film resulted when the slides were exposed to the detergent for less than one minute.

The ability of the solution to remove protein increased in an almost linear manner until 226 p.p.m. available chlorine was reached. Therefore, under the conditions of this study, an optimum level of chlorination was in the vicinity of 200 p.p.m. available chlorine.

A modified diphenylcarbazide method for determining chromium VI in "used" detergent solutions was successful in evaluating loss of metal from stainless steel by action of chlorinated trisodium phosphate. Loss of chromium from type 304 stainless steel, washed with a trisodium phosphate solution for 48 hours at 47° C., was found to be of small magnitude. Even in solutions containing 500 p.p.m. available chlorine, the loss remained negligible. Very little chromium was removed from stainless steel piping cleaned automatically with a chlorinated proprietary alkaline detergent containing up to 234 p.p.m. available chlorine. DETERGENCY OF CHLORINATED TRISODIUM PHOSPHATE ON MILK-PROTEIN SOILS FROM STAINLESS STEEL AND GLASS

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1962

. --- To my wife Phyllis and to my uncle and aunt, Mr. and Mrs. Hardy Merrill, for their invaluable encouragement

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INTRODUCTION

Marked progress has been made in cleaning dairy equipment during the past quarter century. New cleaning agents have been introduced and tinned-copper equipment has been replaced largely by stainless steel.

Chlorinated detergents are one of the more recent introductions. A survey of detergent manufacturers and distributors in the United States revealed that 75 of them were producing or distributing chlorinated detergent compounds. This would indicate that chlorinated cleaners are being used widely and probably to a large extent in the dairy industry. In order of their frequency of use, the major chlorinated compounds were: a) chlorinated trisodium phosphate; b) trichloroisocyanuric acid; and c) dichloroisocyanuric acid.

Often problems and questions accompany the introduction of new products. In the case of chlorinated cleaners the question arises as to the benefits gained through their use. If beneficial, at what concentration do they yield the best results? What is the mechanism by which they accomplish the purpose for which they were designed? Although some data were available answering these questions in part, further studies seemed necessary. To this end the study described herein was undertaken.

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REVIEW OF LITERATURE

Alkaline Detergents

Definition of detergency. According to Niven (1954), detergency is "the removal of any objectionable material from anything and by any means." However, in the dairy industry he limited detergency to a three-component cleaning system involving: a) some liquid, b) some detergency-enhancing material dissolved in that liquid, and c) a mechanical device to facilitate the removal of the soils. Davis (1956) described a detergent as a substance used for cleaning or washing away offensive matter. Lehn (1946), in a closely related discussion, stated that cleaning is the release of contaminating matter from wet surface. The Milk Industry Foundation (1957) considered cleaning of dairy equipment to be the removal of soil from the surface of equipment by dissolving or by suspending the soil in a warm solution of suitable chemicals.

Materials used. The chief detergent materials currently used in the dairy industry are alkaline salts. Claybaugh (1950), Davis (1956), The Milk Industry Foundation (1957), Niven (1954), and many others have confirmed this statement. Also in use in conjunction with these alkaline materials are anionic, cationic, and nonionic wetting agents. These have been discussed by Scales and Kemp (1940) and Sisley and Wood (1952).

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<u>Properties of alkaline detergents</u>. The Milk Industry Foundation (1957) summarizes the common dairy alkaline detergent ingredients and their major properties as shown in Table 1.

The terms used to describe detergent properties have been discussed by Harding and Trebler (1947), Lehn (1946), Little (1947), Mohr and Mohr (1954), Davis (1956), Shogren (1951), Trebler (1945), and others. These are as follows:

- a) <u>Emulsification</u> is the mechanical action of breaking up fats and oils into very small particles which are uniformly mixed with the water used.
- b) <u>Saponification</u> is the chemical reaction between an alkali and an animal or vegetable fat resulting in a soap.
- c) <u>Wetting</u> is the action of water in contacting all surfaces of soil or equipment.
- d) <u>Dispersion</u> implies the disintegration of the soil into smaller particles or droplets and the transport of the soil away from the cleaned surface.
- e) <u>Suspension</u> is the action which holds up insoluble particles in a solution.
- f) <u>Peptizing</u> is the physical formation of colloidal solutions from soils which may be only partially soluble.
- g) <u>Water</u> softening is the removal or inactivation of the hardness of water.
- h) <u>Mineral deposit control</u> is associated with a high dissolving power and a high neutralizing value of the deposits.
- i) <u>Rinsability</u> is the condition of a solution or suspension which enables it to be flushed from a surface easily and completely.

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TABLE 1. - Relative Cleaning Values of Various Detergent Ingredients.

- j) <u>Suds</u> formation is related to foaming and the decrease of surface tension.
- k) <u>Non-corrosiveness</u> is the lack of ability to solubilize equipment with which it comes in contact.

<u>Dairy soils</u>. The requirements of a detergent solution will vary with the extent and nature of the milk deposits (Davis, 1956). According to the Milk Industry Foundation (1957), milk or milk product residues consist of:

- a) Milk sugar, which is readily soluble in water and does not appear to form insoluble products.
- b) Butterfat, which, in its normal, emulsified condition, is readily dispersed in water, but which, when the emulsion is broken, may form a continuous, insoluble film over soluble soil. Fats can be saponified with strong alkalies, particularly at high temperatures, but this method of removal is impractical except in bottle washing and cleaning by recirculation and spraying. Fats in stable emulsions, for example in raw milk, are readily removed by a cold water rinse. Otherwise they are most economically removed by the aid of emulsifying agents at temperatures above the meltpoint of the fat (84° to 97° F.).
- c) Proteins, which when unchanged, as in fresh, raw milk films, are readily dispersed or dissolved in water. Milk proteins are easily changed or denatured by the action of heat or acid and then may no longer be soluble or dispersible in water alone. These denatured proteins may be dissolved readily in dilute alkaline solutions, but do not appear to be soluble in dilute acids. The addition of dispersing agents speeds up the removal of denatured protein films. These agents include dispersive-type wetting agents for use in both acid and alkaline solutions as well as polyphosphates for use in alkaline solutions.
- d) Mineral salts, which in fresh raw milk residues may be readily dissolved or dispersed in water. On the other hand, mineral deposits found in hot milk films or in film left from previous incomplete cleanings or in deposits from hard water

and alkaline materials are usually referred to milkstone. Mineral deposits are slowly soluble in acid solutions with a pH of 5 or less. For rapid solution formation, the pH of the acid must be less than 3. At the lower pH level, the acid is very corrosive and should be kept away from skin and clothing. Dirt, dust, and many other foreign materials usually are removed by the same cleaning procedures which are adequate to remove milk residues, although occasionally special procedures may be required.

More specific reference to dairy soils have been made by many investigators.

Sommer (1938) noted that clarifier and separator slimes were high in milk proteins, fat, calcium phosphates, and other organic matter such as leucocytes.

The composition of milkstone was discussed by Shere (1942), Schwartz (1941), Johnson and Roland (1940), Leeder (1956), Rupple (1960), and Sommer (1938). Sommer (1938) gave the composition of milkstone as indicated in Table 2.

The effects of salt concentration, pH, nature of solvent, added electrolyte, and ionic strength on the solubility of proteins in general, were discussed by Mellanby (1906) and Cohn (1936). A brief summary of their discussion follows:

- a) The solubility of proteins is characteristically minimal at the isoelectric point.
- b) The water solubility of most proteins at their isoelectric points is increased by the addition of small amounts of salt.
- c) The solubility is found to increase with increasing ionic strength, to pass through a maximum, and then to decrease.

Constituent	Minimum	Maximur
	(Perce	ntage)
Moisture	2.66	2.79
Fat	3.63	17.66
Protein	4.14	43.83
Ash	42.03	67.33
CaO	21.05	34.66
P205	17.63	26.93
MgO	1.71	8.12
Fe203	0.0	0.29
Nao	1.40	7.33

TABLE 2. - Extremes in the Composition of Milk Stone*

* Samples from various plants in Illinois, formed on different pieces of equipment under various conditions.

- d) Polyvalent electrolytes are more effective in increasing the ionic strength and are hence more efficient in producing the salting-in effect.
- e) Addition of organic solvents to aqueous solutions of typical proteins ordinarily leads to a reduction in solubility, and usually to precipitation.

McMeekin and Polis (1949) reported that the sedimentation constant of casein was lowered by small amounts of calcium, thus lowering the solubility of the casein.

Little (1947) found that surface and interfacial tensions of detergent solutions were lowered by either neutral or alkaline electrolytes with a resulting improvement in detergency. Clark and Hawley (1958) noted that the stability of hydrophobic sols was due to the presence of an electric double layer. Since the essential charge was frequently the result of adding small amounts of electrolytes, the latter became known as peptizing agents. Similar effects were observed with amino acids, peptides, and proteins.

Physical factors influencing efficiency of deter-

gents on dairy soils. Davis (1956) indicated that the most difficult problem to be overcome by a detergent solution is the cleaning of equipment in which milk has been heated, and particularly in the modern continuous process heattreatment plants.

The albumin and globulin of milk may be denatured by heat (Ramsdell and Whittier, 1953).

Leeder (1956) concluded milkstone formation was reduced by a two-step procedure for heating dairy products. A holding time at low temperature resulted in coagulation of lactalbumin before the high-heating period. Leeder and Martin (1952) reported that the use of a small electric current on the milk pasteurized by high temperature shorttime heating reduced milkstone formation. The device is called the Hydrotron.

During the processing of milk, two types of film may be produced on metal heat-transfer surfaces (Johnson and Roland, 1940). One film was described as cheese-like in composition, and the other film was mineral-like.

The two may be produced concurrently or independently.

In removing milk films, Jennings (1959b) found that kinetics could be applied in establishing the effect of temperature. The reaction between solutions containing hydroxide ion and milk films exhibited a Q_{10} of 1.6. The effect of temperature on the reaction rate was linear, at least between the limits of 36° to 82° C.

Cleaning efficiency was increased by the scrubbing action of occluded air and bubbles under a condition of reduced pressure (Jennings, 1959a). Even without the entrained air, cleaning was more effective under reduced pressure than under higher pressure.

A turbulence-time-temperature-solution concentration relationship was studied by Jennings <u>et al</u>. (1957). Reynolds numbers were used to measure turbulence. In cleaning, turbulence was more significant than temperature. Both temperature and turbulence became less important as the effectiveness of the detergent increased.

Limitations of detergents. In addition to rating poorly on some of the desirable characteristics listed in Table 1, some additional ones have been reported below.

Davis (1956) stated that a detergent solution in use may rapidly change in chemical composition, and physical properties. Among the conditions of use listed were: a) addition of milk constituents; b) dilution by carry-over

of rinse water; and c) absorption of carbon dioxide. The detergent is weakened in proportion to the length of time used. Thus, its efficiency is steadily decreased.

Claybaugh (1950) detected the formation of a chloroprotein film when producers' cans were sanitized with excessive amounts of chlorine just prior to use. This film was extremely difficult to remove by ordinary detergents.

In a study of sanitizers, Cox and Whitehead (1949) observed a similar circumstance in noting that hypochlorites may react with protein deposits on poorly cleaned equipment.

Roland (1942) discussed another undesirable property of many of the alkaline detergent salts. Insoluble precipitates of calcium and magnesium may be formed on equipment when these detergents are used in natural water containing calcium and magnesium ions.

Chlorine

<u>Compounds of chlorine used in detergents and sani-</u> <u>tizers</u>. Reddish (1957) listed the sanitizing compounds as follows:

- a) <u>Hypochlorites</u> are available on the American market as powders containing calcium hypochlorite and sodium hypochlorite combined with trisodium phosphate, and as liquids containing sodium hypochlorite.
- b) <u>Chloramines</u>. The simplest of the chloramines has the formula NH₂Cl.

- c) <u>Chloramine-T</u> is p-toluene sulfondichloramide in which one hydrogen of the amino group of p-toluene sulfonamide is replaced by chlorine.
- d) <u>Dichloramine-T</u> is p-toluene sulfondichloramide in which both hydrogens of the amino group of ptoluene sulfonamide are replaced by chlorine.
- e) <u>Halazone</u> is the name given to p-sulfondichloraminobenzoic acid.
- f) <u>Dichlorodimethyl</u> <u>hydantoin</u> is an organic compound carrying available chlorine.
- g) <u>Succinchlorimide</u> is the chlorinated imide of succinic acid.
- h) <u>Dichlorocyanuric acid</u> is an organic compound carrying available chlorine.
- i) <u>Trichlorocyanuric acid</u> is also an organic compound carrying available chlorine.

Lewandowski (1959) reported on the chlorine materials currently in use as dairy sanitizers. Included were calcium and sodium hypochlorites, di- and trichlorocyanuric acid and dimethyl hydantoin. It is observed that some of the sanitizing chlorine compounds are used in detergent formulations.

A survey of representatives of detergent manufacturers through personal correspondence (Maxcy, 1958; Baskin, 1958; Albrecht, 1958; Bothel, 1958; Yuen, 1958; Leighty, 1958; Ressler, 1958; Barrett, 1958; Wright, 1958; Young, 1958; Zaelke, 1958) indicated the following compounds are the major substances used currently in chlorinated alkaline detergents:

a) Sodium hypochlorite b) Trisodium phosphate

- c) Sodium metasilicate f) Sodium chloride
- d) Tripolyphosphate g) Carbonate
- e) Trichloroisocyanuric acid h) Non-foaming wetting agents

The survey showed that chlorinated trisodium phosphate used alone was the most common detergent mixture of the chemical group.

<u>Chemistry of hypochlorite compounds</u>. As most detergent operations in the dairy and food industries are in an aqueous system, reference is made here to the behavior of hypochlorite compounds in water.

In aqueous solution both sodium hypochlorite and calcium hypochlorite yield hypochlorous acid (Fair, 1948). Hypochlorous acid in aqueous solution can decompose in two ways (Wilson and Bremner, 1948). Their account follows:

- a) 2 HOC1 \implies 0₂ + 2 HCl a reaction slow at ordinary temperatures and much influenced by light and by such catalysts as cobalt salts and
- b) 3 HOC1 = HCO₃ + 2 HC1
 It is this second reaction which is considered
 here. Actually, as hypochlorous acid is fairly
 readily attacked by hydrochloric acid,
- c) HOC1 + HC1 \implies H₂O + Cl₂ Reaction (b) does not occur, but hypochlorites behave similarly and we write
- d) 3 Clo
 cloz + 2 Cl. The reaction is very slow at room temperature but proceeds very rapidly in hot concentrated solutions. It is clear that a three-body collision between anions is most unlikely, but as in many other atom-transfer reactions, the exact mechanism is uncertain. Moderate amounts of free alkali stabilize hypochlorites; the solutions are least stable

at pH 5.7 and have maximum stability at pH 13.1. This supports an older theory that the reaction is between free hypochlorous acid and hypochlorite ion.

e) Clo⁻ + 2 HClO = Clo₂⁻ + 2 H⁺ + 2 Cl⁻ Owing to the small dissociation constant of hypochlorous acid, K = 5.8 x 10⁻⁸, some free acid will always be present. In the absence of free alkali, accumulation of hydrogen ions would cause reaction (c) to take place. Chlorites have been detected in the decomposition products, particularly from alkaline solutions. In considering the reaction, the possibility of hypochlorous acid dissociating in yet another way should be remembered: HOCl - (HO:)⁻ + Cl⁺ The cationic chlorine will be a very powerful

oxidizing agent and may well play an important part in the reaction.

Sommer (1938) indicated an additional method for the decomposition of hypochlorous acid in water:

HOC1 \implies HC1 + 0.

The importance of temperature and pH in the decomposition of hypochlorous acid has already been emphasized. Reaction with organic matter is another property reported by many investigators. The remainder of the review on chlorine chemistry will be discussed under these three factors, namely, pH, temperature, and reaction with organic matter.

In regard to pH, Reddish (1957) gave the percentage of hypochlorite present in the form of hypochlorous acid for various pH values. At high pH the percentage of undissociated hypochlorous acid was very low. The data of Reddish (1957) are listed:

DH	Hypochlorites present as undissociated HOC1 (per cent)
4.0	almost 100.0
6.0	95.8
7.0	69.7
8.0	18.7
9.0	2.2
10.0	0.2

Afanasev (1948) concluded that the oxidizing action of chloramine was due in neutral solution to Clo⁻, and in acid solution to HClO. This conclusion agreed with the data of Reddish (1957), and was supported by Johns (1951b).

The influence of temperature on the bactericidal action of chlorine was studied by Ames and Smith (1944). To disinfect water containing <u>Escherichia coli</u> with 0.25% organic nitrogen present, the time required at 8° C., was more than nine times that required at 40° C. for a given chlorine level.

Reddish (1957) reported data indicating that in the range of 20° to 50° C., the killing time of <u>Bacillus</u> <u>metiens</u> spores by calcium hypochlorite was reduced 46 to 66% for each 10° C. rise. Increased rate of diffusion of the germicidal agent (undissociated hypochlorous acid) was believed to be responsible.

The reaction of hypochlorites with organic matter has been studied by many investigators. Theoretically, the action of free halogens on protein in aqueous solution can take any of three paths: a) addition, b) substitution,c) oxidation (Herriott, 1947).

Wright (1926, 1936) concluded that hypochlorites normally react with amino acids and proteins to form (if only as unstable intermediate products) typical chloramino compounds. Cox and Whitehead (1949) corroborated the findings of Wright. Faber (1947) suggested that a decrease in the oxidation-reduction potential may be used to measure the chemical activity when hypochlorous acid combines with organic matter.

Lasmanus and Spencer (1953), Haller <u>et al</u>. (1941), and Wilkowske and Krienke (1955) found that milk dissipated the hypochlorous acid in chlorine sanitizing solutions.

The presence of ammonia also decreased the effectiveness of sanitizing solutions containing hypochlorous acid (Van Hall, 1954; Weber <u>et al.</u>, 1940).

Cahn and Powell (1954) reported that nitrogen was liberated when ammonia and hypochlorite were allowed to react in an alkaline medium.

Herken and Siebersiepe (1953) reported that the peptide bonds of several albumins and globulins were hydrolyzed by hypochlorites.

Materials other than proteins appearing in dairy soils may react with chlorine compounds. Lyness and Quackenbush (1956) found that methyl oleate, oleic acid and ethyl linoleate gave near quantitative yields of

addition products when treated with chlorine. Zoller (1923) concluded that other substances present in the milk were more easily attacked than the fat after observing that the presence of 3.8% butterfat did not affect the decomposition rate of sodium hypochlorite in milk.

Benefits of chlorine compounds as detergent aids. A comparatively recent development in cleaning has been the use of chlorinated detergents in the food industries. Hiscox and Briggs (1955) and Holland <u>et al.</u> (1953) reported beneficial cleaning effects from chlorinating alkaline detergents. Lewandowski (1959) stated the addition of sodium or calcium hypochlorite, or organic compounds bearing chlorine, to a well-balanced alkaline cleaner gave brighter surfaces and reduced the tendency toward filming. Lewandowski emphasized that these chlorine compounds will not clean well when used independently of alkaline detergents.

In a laboratory study, MacGregor <u>et al</u>. (1954) recommended adding 25 to 100 p.p.m. sodium hypochlorite to certain alkaline detergents for improving the protein cleanability of metal surfaces.

Kaufmann and Tracy (1959) found chlorinated alkaline detergents of varying value in removing an iridescent discoloration from cleaned-in-place pipelines.

Shepard (1960) and Overman (1959) mentioned the brightening effect noted by Lewandowski (1959). However, both of these investigators and Rupple (1960) agreed that the major benefit of chlorinating the detergent was to improve peptization. Other benefits listed by Overman (1959) were:

- a) Changing the electro-static balance of the cleaning solution so that the soil which would normally be attracted by and adhere to the surfaces becomes free rinsing. Utensil and machine odors were largely eliminated.
- b) Reduction in foaming of the washing solution.

Corrosion by chlorine-bearing compounds. Botham (1949), Foster (1949), Jacobsen (1954), Johns (1951a), Mitten (1954), and Myrick (1954) were a few of the many in-Vestigators who noted the corrosive properties of chlorinebearing compounds under certain conditions of use.

Of these authors, Botham (1949) and Johns (1951a) Stated that corrosiveness was inversely proportional to Alkalinity, and directly proportional to temperature, time, and concentration of available chlorine.

Jacobsen (1954) considered that corrosion by chlorine disinfectants was negligible when used in concentrations of 100 and 200 p.p.m. as is normally done in dairy processing plants.

Klenzade Products, Inc., (1960) stated that the corrosiveness of compounds containing chlorine may be modified by increasing the pH. Addition of sodium hydroxide or sodium silicate was recommended.

Evaluation of Corrosion

of Food Processing Equipment

Mitten (1954) has described the nature of corrosion. A portion of his discussion, which follows, summarizes briefly the principle of corrosion and some of the pertiment terms.

> Metals and their alloys tend to revert to one or more forms in which they are found in their natural ores. Eventually they will do this. Man has discovered no way to prevent this earth-toearth return. By skillful metallurgical processes and by proper care, man can prolong the process by slowing the rate of corrosion, but he cannot completely prevent it.

Corrosion is an electrochemical process which destroys the metal or alloy and (1) non-metallic solids such as sulfur, (2) liquids such as acids or water, or (3) gases such as chlorine and air.

In the most common form of corrosion by the electrochemical process, there is generally an anode, a cathode, and an electrolyte. By an exchange of electrons, atoms of metal and ions in the solution (usually hydrogen ions) replace each other. Ions are atoms having an electrical charge. When the metal atoms and solution ions replace each other, hydrogen gas evolves or a second chemical change takes place so that hydrogen and oxygen combine to form water. The metal particle then goes into solution. As this is repeated millions of times, the metal surface is corroded away. Some of the factors in corrosion are acidity of solution, aeration and oxidizing agents, temperature, velocity, and effect of films.

Uhlig (1948) listed the methods of determining the amount and influence of corrosion as:

- a) Visual observation
- c) Changes in electrical resistance
- b) Change in weight
- d) Oxygen evolved
- e) Depth of pitting
- i) Electrochemical
- f) Hydrogen evolved j) Electrometric
- g) Microscopic

- k) Optical methods
- h) Changes in physical properties

Use of specific methods for evaluating corrosion has been reported by many investigators. Holland et al. (1953) have judged corrosion visually in a long-term study on cleaned-in-place stainless steel pipelines. Botham (1949) combined visual evaluation with measurement of depth Of pits resulting from corrosion. Electron diffraction, -ray diffraction, and electron microscopy have been used by Yearian et al. (1957) in determining the formation of Oxide films on 18-8 stainless steels. These steels were Oxidized in air at 300 to 700° C. By electron diffraction Studies Kaufmann et al. (1955) found the composition of iridescent film on stainless steels to be metal oxides. Carius (1956) followed the course of passivation of nonrusting steels by recording changes of potential. The Steels were exposed to solutions and the variation in potential observed. Changes in weight of test samples have been the most common technique aside from visual observation in detecting corrosion of dairy metals. The gravimetric technique has been utilized by Barnum et al. (1937), Finley and Foter (1947), Haller et al. (1941), Mohr and Wortman (1953), Fluette (1960), and Klenzade Products, Inc.,

(1960). Harper (1958) showed close agreement between weight changes and a radiometric method using P³² on type 302 stainless steel.

Methods of Evaluating Cleaning

Davis (1956) emphasized that the only completely satisfactory way of testing detergents and methods of cleaning is to try the method and the detergent under the existing conditions for the cleaning operation concerned. He acknowledged various laboratory procedures which have been suggested for testing the efficiency of detergents. He reported these can be very useful provided their limitations are always appreciated. Davis felt it was usually unwise to draw hard and fast conclusions from such results.

The majority of methods to evaluate cleaning fall into two general areas. One is the assessment of viable bacteria present after the cleaning operation. The other is the determination of the amount of soil retained on a surface after cleaning. Most investigators have used some combination of both of these principles.

Bacon <u>et al</u>. (1953) used a bacterial swab technique to show the cleanliness of beer glasses, Fleischman and Holland (1953) for glass pipelines used for milk, Hucker (1954) for surfaces of eating utensils, Jensen <u>et al</u>. (1959) for recirculation-spray versus manual cleaning of milk storage tanks, Kaufmann <u>et al</u>. (1955) for in-place-cleaning of stainless steel lines, and Kaufmann <u>et al</u>. (1960) for comparing cleanability of various stainless steel finishes. Kaufmann <u>et al</u>. (1960) also used the bacteriological techniques of the Direct Agar Contact Plate, and the Direct Surface Agar Plate Test for comparing the cleanability of various stainless steel finishes.

In testing the efficiency of detergents under factory Conditions by microbiological methods, Meewes (1952) exannined the relative merits of plate counts of emulsions, impression plates, and thin layers of agar on the treated Surface.

The capacity of surfaces to retain soil, a second principle of evaluating cleaning, has been assessed in many ways.

Calbert (1958) and Lewandowski (1954) reported re-Sults obtained by gravimetric methods after investigating the cleanability of cleaned-in-place pipelines, and milkstone formation, respectively.

Measurement of light transmission through soiled glass surfaces was the technique used by: a) Jensen (1946) in determining the function of detergents employed in cleaning dairy equipment as affected by various detergents and procedures, b) Claybaugh (1950) in his investigations of detergency applicable to mechanical can washing, c) Mead and Pascoe (1952) for assessing the cleaning efficiency of detergents, d) Leenerts <u>et al</u>. (1956) in soil removal

by dishwashing detergents, e) Gilcreas and O'Brien (1941) for evaluating detergents and methods of cleaning eating utensils, and f) Hughes and Berstein (1945) in studying compounds used in dishwashing machines.

Radioisotopic techniques now exist which are reported to avoid the pitfalls of the light transmission methods. The "light" disadvantages named, were low sensitivity at both high and low levels of soil residues (Masurovsky and Jordan, 1958). The same investigators (1960) found that the procedure involving the determination of <u>Staphyloccus aureus</u> labeled with isotopes was superior to macro-colony agar submersion counts, and Bacto-Strip determinations for measuring the cleanability of milkcontact surfaces. Hays <u>et al</u>. (1958) and Hays (1960) used <u>Escherichia coli</u> labeled with P³² in studying the cleanability of surfaces in contact with dairy products. Ridenour and Armbruster (1953) utilized organisms labeled with P³² in an analysis of the cleanliness of eating surfaces.

Cucci (1954) labeled milk with P^{32} to measure the amount of milkstone deposited on rubber, Pyrex glass, and Tygon tubing. Seiberling and Harper (1956) found that P^{32} was unsatisfactory for labeling milk films in studying the cleanability of cleaned-in-place automatic valves. They reported that P^{32} reacted irreversibly with stainless steel and therefore preferred Ca⁴⁵ for evaluation of cleaning.

These same investigators found traces of Ca⁴⁵ on surfaces shown to be bacteriologically clean. Thus, the isotopic procedure was more sensitive in detecting soil than the bacteriological method.

Extensive use of homogenized milk labeled with P³² has been made by Jennings (1959a, 1959b), and Jennings et al. (1957). Criticism of the method involving the labeling of milk with P³² was made by Peters and Calbert (1960) who Compared the relationship between the weight of soil removed from test films and the decrease in radioactive count. Peters and Calbert labeled milk by three methods. One was **by** direct incorporation of P^{32} into the milk used to make the test soil. The second method involved the inoculation **Of** P^{32} into the blood stream of a goat. When these two methods were used to label milk there was an unsatisfactory agreement between loss in weight of the soil from the test films and the decrease in radioactive count. However, the third method of labeling milk, namely of incorporating bacterial cells labeled with P³² into the test soils, gave good agreement with the gravimetric method.

Recently, Jennings (1961) reported a statistically controlled study in which 240 samples of milk labeled with P^{32} were used. Following a detergent treatment there was good correlation between methods involving determination of weight loss from films and decrease in radioactive count. According to Jennings, differences in the preparation of

test films were responsible for a lack of correlation between losses of film measured by gravimetric analyses and by an isotopic method involving the direct labeling of milk with P³². He also reported a good correlation between the gravimetric method and a procedure in which labeled bacteria were incorporated into milk films, but indicated the latter required more effort in preparation.

Many workers have made qualitative visual measurements of cleaning, either independent of, or in combination with, one of the above described quantitative techniques.

Specifically, visual evaluation has been utilized by Jensen and Waterson (1950), Jensen <u>et al</u>. (1959), Kaufmann <u>et al</u>. (1955), Kaufmann and Tracy (1959), Wildbrett <u>et al</u>. (1954), Fleischman and Holland (1953), Holland <u>et al</u>. (1953), Hucker <u>et al</u>. (1951), Mohr and Junger (1953), Mohr, Junger, **and** Weinert (1953), and Mohr, Weinert, and Peters (1953).

In determining the effects of several physical properties of detergents on cleanability, a few of the many interesting techniques should be noted: a) Mohr and Mohr (1954) determined wettability of detergents by measuring the contact angle of the detergent solutions--no relationship was found between detergency and surface tension; b) Lindquist (1953) devised a method of eliminating obviously unsuited wetting agents by recording, photographically, the dimensions of created drops, dimensions of the drops were then used with a formula to calculate surface tension--by

this method, wetting agents which foamed excessively were detected by comparison with standard products showing desirable foaming characteristics; c) Bisio <u>et al</u>. (1956) reported on the feasibility of recording the spreading rate of a drop of oil on a metal surface and using this measurement as an indication of cleaning efficiency; d) Jennings (1959a) has successfully used Reynolds numbers to study the turbulence factor in cleaning; and e) Mohr <u>et al</u>. (1952) **Buggested** a special glass electrode containing lithium for determining pH of highly alkaline detergent solutions.

Creation of Synthetic Soil Films

Synthetic films for detergency studies. The results of studies concerned with the removal of films of soil have been discussed in a former section. Mention is now made of Various representative methods used to create synthetic films for detergency investigations. It will be noted that air drying, heating, and addition of various chemicals have been used widely to make the film materials tenacious.

As previously mentioned, milk films have been made radioactive directly with P^{32} or indirectly with bacteria labeled with P^{32} . Such films have been prepared by Cucci (1954), Jennings <u>et al</u>. (1957), Masurovsky and Jordan (1958), and Hays <u>et al</u>. (1958, 1960).

Cucci (1954) employed homogenized milk labeled with P^{32} to fill sections of rubber, Tygon, and Pyrex tubings.

The milk was removed after 24 hours and the tubings allowed to air dry before testing the detergent.

Jennings <u>et al</u>. (1957) placed 0.5 to 1.0 ml. of labeled homogenized milk in the center of flat disks of stainless steel which were of 15/16 in. diameter. The disks were then steamed to dryness.

Pasteurized homogenized whole milk or sterile 0.85% saline solution was tagged with radioactive cultures of \underline{E} . <u>coli</u> or <u>Micrococcus pyogenes</u> var. <u>aureus</u> by Masurovsky and Jordan (1958). The inoculated soil (0.05 ml.) was pipetted onto the center of 1 in. diameter disks of various materials used as contact surfaces. After mechanically spreading the soil, the disks were dried for 6 minutes at 60° C. with an infra-red lamp and 10 minutes at 80° C. in an oven.

Hayes et al. (1958, 1960) spread 0.4 ml. of cream, buttermilk, homogenized milk, or chocolate milk inoculated with <u>E. coli</u> evenly over one side of plastic and stainless steel disks 2 inches in diameter. The films were then air dried.

Beef and mutton fats, butter, skimmilk, egg, peanut butter, lard, and evaporated milk, alone or in mixtures, were manually spread over china, and plastic surfaces by Hucker <u>et al</u>. (1951) and Hucker (1954).

MacGregor <u>et al</u>. (1954) manually distributed a slurry containing CaCO₃, CaHPO₄ • 2H₂O, spray-dried milk milk powder, and distilled water on stainless steel strips. Drying of the films was accomplished by autoclaving for 25 minutes.

Jensen (1946) and Claybaugh (1950) manually immersed 3 in. glass squares, 1/8 in. thick, in raw milk and followed this with additional treatments. Air-dried raw milk films were prepared by dipping the glass squares twice into raw milk held at 40 to 50° F. After a 15-minute draining and drying period. the dipping was repeated several times according to the amount of film needed. Heat-treated raw milk films were made as above except that these films were heated at 180 to 185° F. for 15 minutes after the air drying, but before the next dipping. Five complete sequences of dipping, drying, and heating were used to give the finished heat-treated film. In making "chlorine-protein complex" films, the glass squares were dipped twice in raw whole milk, drained, and air dried for 15 minutes. The glass was then immersed in a 250 p.p.m. NaOCl solution, and air dried for 15 minutes. Five complete sequences of this procedure completed the "chlorine-protein complex" film.

Gilcreas and O'Brien (1941) prepared a synthetic wash- and rinse-water of known hardness, and deposited this on glass microscope-slides. Calcium chloride and magnesium chloride was added to a 0.3% detergent solution in distilled water. The ratio employed was six parts of calcium hardness

to four parts of magnesium hardness, each expressed as calcium carbonate. To simulate a milkstone film, the slides were dipped in this mixture, drained 3 to 5 minutes, and sometimes heated at 110° C. for 3 minutes.

By means of an Autotechnicon (Lewandowski, 1954), weighed stainless steel strips were dipped consecutively into a chlorine solution (200 p.p.m.), raw milk, water, detergent, and water. The water contained 300 p.p.m. hardness. This basic cycle was repeated 30 times. Various methods of heating were used to dry the slides.

<u>Surface-film techniques utilized in other scientific</u> <u>fields</u>. Some surface-film techniques developed in other fields were adopted for use in this investigation.

Rothen (1956) reviewed the results made possible by using surface-film techniques. Included were determinations of chemical and physical properties such as film formation, surface pressure, molecular weight, viscosity of surface films, film compressibility, and film thickness.

Blodgett (1935) built films by transferring monomolecular layers of various metallic soaps and fatty acids from a water surface to several solid surfaces. The thickness of "step" films was measured by means of the interference of reflected monochromatic light. In similar work, Langmuir <u>et al</u>. (1937) suggested that variations in thickness of much less than 10^{-8} cm. can be observed using optical means. Blodgett (1937) reported that the process of building films with substances such as proteins, required that the solid surface be lowered or raised through a water surface on which the film is spread.

The importance of a mechanical slow-dipping device in producing uniform films was discussed by Dean <u>et al</u>. (1939).

Joly (1939) stated that the surface viscosity of a protein film varies according to the care taken at the time of its spreading.

It was believed by Langmuir (1938) that protein films were insoluble or denatured because hydrophilic groups were drawn into the liquid surface, distorting the molecule, and submerging hydrophobic groups which normally occupy an internal position in the molecule. Langmuir suggested that methods of surface chemistry were of value in studying specific protein reactions.

Absorbed films of proteins at air-water, and oilwater interfaces were investigated by Alexander (1951). Using the surface viscosity method, his studies showed that the thickness of absorbed films of proteins depends upon such factors as time, the nature of the interface, pH, salt concentration, and temperature.

Braude and Nachod (1955) showed that multiplereflection interferometer-methods made possible the direct determination of single monolayers deposited on solid surfaces.

A comprehensive review of gravimetric, photometric, interferometric, x-ray absorption, radioactive tracer, and electrical methods for measurement and control of the thickness of thin films was made by Greenland (1952).

It was recommended by Gunn (1946), that in measuring films, the films be arranged to cover only a part of the plate. This made possible, at some position on the surface, an abrupt step equal to the thickness of the film.

Lucy (1948) studied surface films by the reflection of polarized light. He noted that plane-polarized light is generally polarized elliptically by reflection. Since the molecules in a very thin layer are those chiefly concerned in the process of reflection, the ellipticity is very sensitive to the conditions at the interface, such as the presence of films.

Rothen and Hanson (1948) used the ellipsometer to measure the thickness of transparent films, from one to many thousands of Angstrom units, deposited on polished metal slides.

Diphenylcarbazide Method for Measuring Chromium VI

Cazeneuve (1900) and Moulin (1904) were among the first to use diphenylcarbazide in estimating minute amounts of chromium colorimetrically. Sandell (1944) reported that diphenylcarbazide gave a clear red-violet color when reacted with hexavalent chromium.

Detergent solutions, after use in the dairy and food industries, may be regarded as biological systems because of contamination with the various soils. In studies on biological materials, diphenylcarbazide has served to detect chromium in blood (Cahnman and Bisen, 1952); in water, sewage, and industrial wastes (American Public Health Association, American Waterworks Association, and Federation of Sewage and Industrial Wastes Associations, 1955); in urine, tissues, and blood (Urone and Anders, 1950); and in urine (Saltzman, 1952).

Chemically, diphenylcarbazide is formed by the condensation of urea and phenylhydrazine (Udy, 1956). According to Welcher (1947), diphenylcarbazide has the formula $C = (NH - NH - C_{6H_5})_2$, a molecular weight of 242.27, and is a white crystalline solid. It is very slightly soluble in Water and slightly soluble in hot alcohol, acetone, and glacial acetic acid. In acid solution, the diphenylcarbazide method is nearly specific for hexavalent chromium; molybdenum is the only element giving a similar, but less sensitive, violet color (Sandell, 1944).

Morrison and Freiser (1957) reported that microgram quantities of hexavalent chromium can be evaluated by the diphenylcarbazide method. Saltzman (1952) found a recovery of 98% on 50 ml. portions of urine containing 0 to 13 µg. of chromium. The American Public Health Association, American Waterworks Association, and Federation of Sewage and Industrial Wastes Associations (1955) recommended the method be performed on samples having 10 to 100 µg. of chromium.

Many of the factors influencing the reliability of the diphenylcarbazide method have been studied and reported (Davis and Bacon, 1948; Feigl, 1946; Rowland, 1939; Sandell, 1944; Welcher, 1947; Saltzman, 1950; Cahnman and Bisen, 1952; Brookshier and Freund, 1951; Urone, 1955; Urone and Anders, 1950).

Cahnman and Bisen (1952) stated the main problem in applying the diphenylcarbazide method to biological materials, such as blood, was in eliminating the numerous factors interfering with the precision of the method. The factors noted were: a) incomplete oxidation of trivalent to hexavalent chromium; b) presence of minute amounts of reducing agents; c) loss of chromium through volatilization; and d) interference of heavy metals.

Sandell (1944) reported the interferences of the heavy metals, Mo^{VI} , Fe^{III} , Hg^{II} , and V^{V} , could be eliminated by employing a green filter (Cenco No. 2) on the spectrophotometer.

Brookshier and Freund (1951) described a method for separating chromium from vanadium interference.

If the ratio of molybdenum to chromium was less than 10, Rowland (1939) reported molybdenum interference could

be neglected.

Urone and Anders (1950) noted manganese as permanganate gave a faint yellow color with diphenylcarbazide. Sodium azide was recommended to reduce the permanganate before addition of the diphenylcarbazide.

Feigl (1946) suggested heavy metal interference could be prevented by the addition of suitable compounds which reduced the ionic strength below that required for the diphenylcarbazide reaction.

Development of color with diphenylcarbazide was shown by Davis and Bacon (1948) to be slower in phosphoric acid than in sulfuric acid. However, full color developed within five minutes in acidities up to 2N. Welcher (1947) reported the optimum acidity was 0.2N. At lower acidity the color development was slow, and at acidities greater than 0.2N the color was less stable.

According to Saltzman (1950) ashing was generally required to destroy reducing materials such as organic matter. Saltzman also reported that polyphosphate complexed chromium making it unreactive to permanganate oxidation. Phosphate was converted to polyphosphate by strong heating and followed the reaction:

2 NaH₂PO₄ \rightleftharpoons Na₂H₂P₂O + H₂O

This reaction was also found to occur to some extent when a wet ashing solution of nitric acid was taken to dryness following the ashing. In eliminating nitric acid after

ashing, Saltzman recommended low temperature evaporation on the steam bath. In this manner, the nitric acid was kept at 100° C. and the conversion of phosphate to polyphosphate prevented.

Urone (1955) found the sensitivity of the diphenylcarbazide reagent varied inversely with the amount of discoloration developed. Therefore, Urone recommended discarding solutions which had discolored with age.

EXPERIMENTAL PROCEDURE

Measurement of Soil Retaining Capacity

Measurement of soil retention was studied by means of the a) ellipsometer, b) interferometer, c) electron microscope, and d) gravimetrically. H. J. Trurnit of Rias, Inc., Baltimore, Maryland, and A. Rothen of the Rockefeller Institute in New York City performed the ellipsometer investigations in this study. Trurnit (1959) used a Rudolph ellipsometer (Model 441) and followed a method of his own design. Stainless steel slides of No. 4 and No. 7 finishes were coated mechanically with 19 double layers of barium stearate followed by 10 single additional layers of the same substance. Each layer was approximately 50 A. thick and 1/8 in. wide.

Rothen (1959), another cooperator, also used a Rudolph ellipsometer (Model 200E) in measuring the thickness of films of pasteurized skimmilk soil deposited on similar stainless steel slides as well as on glass microscope slides. Both sides of the glass and steel slides were coated with film by dipping the slides mechanically into skimmilk. All filming in this study was performed mechanically as indicated. The films covered the entire width of the slide and a length of approximately 40 mm.

The interferometer measurements were made on the instrument in the Michigan State University Department of

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Physics and Astronomy. Dr. T. H. Edwards of that department supervised the work and cooperated in the study. The apparatus consisted, in part, of a light source, "light box," and an "optical flat." A General Electric sodium vapor lamp served as the source of monochromatic light. This light passed into an adjacent light box through a diffusing glass. Here, the light struck a plain glass panel angled at 45°. The incident light was thus bent 90° and directed perpendicularly to the surface of an optical flat. A nonreflecting background was obtained by placing black velvet under the optical flat.

The glass slides were dipped once into the skimmilk, then air dried and placed on top of the optical flat. The slide was manipulated by hand to give the best visual observation of the displacement in the interference lines caused by the milk films.

The electron micrographs of milk films were taken by Bass (1960) at a magnification of 8,000 to 10,000 using an R.C.A. electron microscope (Model 2C). The procedure was as follows: Clean, clear, glass, microscope slides were sprayed with very dilute, atomized droplets of nitrocellulose, and allowed to air dry. Amyl acetate was used to dilute the nitrocellulose. One mechanical immersion of the slide in skimmilk served to apply the skimmilk film which was allowed to dry in air. The slide was then dipped once in a 1% solution of nitrocellulose in amyl acetate and air dried. The last film of nitrocellulose, when lifted away from the milk film surface, tore away some of the original atomized droplets of nitrocellulose. This produced a "replica" of the milk film surface, as well as showing its depth in the areas of the nitrocellulose droplets. The replica was shadow cast with a Kinney (Type SC-3, 0.T.) high vacuum metal evaporator. Electron micrographs were then taken which revealed the surface topography of the film and its thickness.

The gravimetric method consisted of weighing slides, filming these slides with skimmilk, and reweighing to obtain the weight of the film.

Clear, unetched, glass microscope slides were handled with forceps and cleaned chemically by submerging in a sodium dichromate cleaning solution for 24 hours in covered, glass, slide-staining dishes. All rinsing of the slides was done in the staining dish using running distilled water. After rinsing, the slides were submerged for one hour in concentrated sulfuric acid, the initial temperature of which was 60° C. Following another thorough rinsing with distilled water, the slides were placed end up on paper towels in a 60 to 70° C. oven until used.

Number 4 finish stainless steel sheets were made into slides having dimensions of 75 x 25 x 2 mm. These slides were cleaned by suspending them in a 4-1. beaker from clamps attached to a circular rack. The beakers

alternately contained hot solutions of proprietary organic acid and alkaline cleaning materials. Agitation of the solutions was performed by a magnetic stirrer. After thorough rinsing in running distilled water, the steel slides were handled and stored by procedures similar to those for the glass slides.

The clean steel and glass slides were weighed before and after filming on a four-place Voland balance (Model 750-D). Comparisons of accuracy were made between the Voland balance and a Rueprecht analytical-type balance allowing estimation of a five-place weight. Before weighing, slides and films were allowed to come to equilibrium with the atmosphere for one hour. While in storage, the filmed slides were protected from dust contamination by placing them in a large covered glass vessel. To minimize damage to the films, forceps and light metal storage racks were employed during the weighing and storage periods.

The filming material consisted of skimmilk tempered as the experiment dictated. Various films were obtained by dipping the slides a variable number of times into the skimmilk.

The filming apparatus used is shown in Figure 1. The only variation made in this equipment throughout the study was the use of either a 4-1. Pyrex beaker or a oneliter Pyrex crystallizing dish to hold the dipping solutions. Slide racks were constructed by welding 1/2 in. slices of 4 in. steel pipe to ring-stand clamps. Six to ll ball-joint clamps were bolted around the periphery of the slice of pipe equidistant from one another. These clamps were designed to hold the glass and steel slides for the filming procedure. After placing the constructed slide racks on a ring stand, the racks were heated and adjusted to be in plane with a horizontal surface.

The slides were aligned in the rack to allow equal filming depth. Alignment was accomplished by placing the ring stand with its attached rack and slides on a 1.25 ft. x 2 ft. x 1/2 in. clean "desk" glass. The slide rack was adjusted on the ring stand at a predetermined height. The slides were then manipulated in the clamps so that their lower edges coincided fully with the flat surface of the "desk" glass.

A device designed to dip at a constant rate was made by modifying a Raytheon curd tension meter (Model 2-505) to permit the attachment of the slide racks. Adjustable base supports allowed leveling of the complete dipping assembly. A plumb bob suspended from the center of the slide rack acted as a gauge for dipping depth. Reproducible dipping depths were attained by manually switching the machine to reverse when the plumb bob reached an arbitrary depth in the dipping medium. The filmed slides were heated in an air circulated oven for varying

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times and temperatures before reweighing.

Methods of Evaluating Cleaning

Two methods, the Spectrophotelometer and the microkjeldahl, were used to determine the effectiveness of soil removal from the slides. The Spectrophotelometer methods of Jensen (1946) and Claybaugh (1950) were used with some modification.

Essentially, the method consisted of a) creating films on glass microscope slides, b) measuring the percentage light transmission, c) subjecting the films to a controlled washing treatment, d) remeasuring the percentage light transmission, and e) then evaluating the change in percentage transmission resulting from the cleaning treatment.

In addition to the skimmilk used to film the slides as described previously, a 0.3% solution of detergent mixture (w/v) in distilled water was used as a filming material. The detergent mixture was composed of 45% Na_3PO_4 , 49% Na_2CO_3 , and 2% Vel which is an anionic wetting agent. Six g. of calcium chloride and 4 g. of magnesium chloride (MgCl₂·5H₂O) were added to each 4-1. portion of the distilled water.

The correctly aligned racks of slides were successively dipped once in skimmilk, air dried, and oven dried. Air drying was facilitated by shielding a round hot

plate with a paper device and suspending the slide racks inside this shield. The air drying was continued until no visual moisture was present. Oven drying during film making consisted of suspending the slide racks in a 70 to 80° C. air-circulated oven for 15 minutes. At the completion of the filming procedure oven drying was accomplished by heating the films for 15 to 16 hours at 70 to 80° C.

Controlled washing consisted of suspending the films from their racks in magnetically stirred, 4-1. Pyrex beakers of detergent solution. A 24-1. capacity Blue M Electric (Model MW 1130) water bath furnished sufficient capacity for controlling the temperature of both the distilled water and the 4-1. Pyrex beakers.

In preparation for the washing procedure, the beakers were removed from the water bath and filled to the 4-1. level with the heated distilled water. Sufficient detergent was added to give the desired concentration. Although the temperature of the detergent solution was not controlled, a maximum drop of 1° C. resulted during the washing procedure which required three minutes.

The percentage of light transmission through the filmed slides before and after washing was determined by a Cenco Sheard Spectrophotelometer. The filmed slides were placed in the light path in a position normally occupied by the filter, and readings were taken on three different areas of the slide. The three representative areas

selected for making readings on one slide were in the same positions as those used for all other slides. An arithmetic average of the three readings was recorded. The same clean microscope slide was used to adjust the percentage transmission to 100 before reading each slide. A one-hour warm-up period before using the apparatus minimized the amount of adjustment necessary.

The microkjeldahl technique for evaluating residues of film remaining after washing involved several specific procedures which are described below. The essential equipment for this method of forming film employed the device shown in Figure 1, combined with a microkjeldahl apparatus for nitrogen determination. When desirable, the temperature of the washing solutions could be controlled as shown in Figure 2. Micro cover glasses ($22 \times 50 \text{ mm.}$) were used as the film supports. For the purpose of determining sample size, the micro cover glasses were weighed on a Christian Becker (Style AB-2) analytical balance before and after filming.

Several materials in different combinations were used to create the films. After heating to various temperatures for extended periods, the films were given a controlled washing without agitation. Following the washing the two filmed micro cover glasses, comprising an individual sample, were "quantitatively broken" into a 50 ml. microkjeldahl flask with the aid of sharp pointed forceps.

Figure 2 shows the position of the unbroken slides in the neck of the flask. A nitrogen determination was performed on the film residue remaining on the cover glass after the washing period. Four milliliters of concentrated sulfuric acid were used to coat the film residues immediately after the washing period but prior to breaking them into the microkjeldahl flask. Each flask contained two glass beads and approximately 0.5 g. of potassium sulfate (K_2SO_4). clean glass rod was used to push the glass fragments down into the bulb of the kjeldahl flask and to crush the larger glass fragments to facilitate complete oxidation. One ml. of 10% copper sulfate (w/v, aqueous, $CuSO_4$) in a 5 ml. hypodermic syringe was used to wash the glass rod before its extraction from the flask. Nitrogen determinations were by the method of Luecke (1960). Results were expressed as µg. of nitrogen per mg. of film sample.

Methods of Evaluating Corrosion of Stainless Steel

The corrosion of stainless steel was measured by a) conductivity, b) emission spectroscopy, and c) diphenylcarbazide method for chromium VI.

Measurements were made comparing the difference in conductivity between hypochlorite solutions exposed and not exposed to stainless steel surfaces. An Industrial Instrument conductivity bridge (Model RC 16) with a 2 ml. cell, having a cell constant of 0.8500, was used in the

tests. Five 75 x 25 mm. stainless steel slides were washed collectively in four liters of distilled water. The washing apparatus was identical with that previously described. Sufficient hypochlorite, Klenzade XY-12, was added to the 4-1. portion of distilled water to give 44 p.p.m. available chlorine.

The available chlorine was determined by the starch iodide titration method (Milk Industry Foundation, 1959). The chlorinated solutions were held at 45° C. during the respective 5-, 10-, and 15-minute exposures to the stainless steel. Conductivity was determined by the method of Brunner (1960) who used the procedure to obtain identical conductivities of protein and buffer solutions prior to electrophoretic studies.

The emission spectroscopy analysis for iron was performed on chlorinated trisodium phosphate solutions after exposure to 1120 sq. cm. of the surface of type 304 stainless steel beakers with a capacity of 7-1.

Three Vollrath 7-1. stainless steel beakers, bearing the factory identification number 7878, were used as the test surfaces. Four liters of triple distilled deionized water were added to each beaker. Addition of 12 g. of technical grade trisodium phosphate $(Na_{3}PO_{4})$ gave a 0.3% solution (w/v). Sodium hypochlorite (NaOCl) was added to the phosphate solutions in varying amounts. One beaker contained no NaOCl, the second 42 to 49 p.p.m. available

chlorine, and the third 431 to 454 p.p.m. available chlorine. Each phosphate solution was exposed to the stainless steel surface at a temperature of 47° C. for 48 hours. Temperature control was accomplished by placing the three 7-1. beakers in the 24-1. water bath previously described. Continuous and uniform agitation was furnished by three electric continuous stirring devices having propeller-type agitators. Evaporation losses were minimized by using heavy cardboard fiber circles as covers for the steel beakers. Evaporation losses from the original 4-1. volume were restored as necessary by adding triple distilled de-ionized water.

At the end of the 48-hour exposure period, the phosphate solution in each beaker was quantitatively transferred to a clean 4-1. Pyrex beaker for evaporation. Without boiling, the 4-1. volume was reduced to 100 to 150 ml. over a low temperature hot plate. These evaporated solutions were, in turn, washed quantitatively into Berselius, tallform 200 ml. Pyrex beakers. Further low-temperature evaporation reduced the volume to approximately 10 ml.

A modification of the "8-Quinolinol" extraction procedure for iron (Morrison and Freiser, 1957) was used to separate iron from the large amount of sodium present in the samples. The steps used were:

> a) Acidify the 100 ml. evaporated sample to approximately pH 5 with 1:1 HCl (v/v, aqueous).

- b) Filter the sample through Whatman filter paper (No. 42) into a clean 400 ml. separatory funnel.
- c) Add 10 ml. of 1% 8-quinolinol (w/v) in chloroform.
- d) Shake 1 minute and let stand 10 minutes.
- e) Draw off the bottom layer (chloroform) into a 200 ml., Berselius, tall-form Pyrex beaker.
- f) Repeat the extraction twice more using 5 ml. of 8-quinolinol for the last extraction.
- g) After final decanting, evaporate the solution to dryness on a steam bath.

The samples were then examined spectrographically for iron according to the procedure of Bass (1960) which is briefly described in the following outline:

- a) Ash the previously evaporated samples at 490 to 500° C. for 30 minutes in a muffle furnace.
- b) Add an internal standard consisting of 1 ml. of 2.4% CoCl₂·6H₂O (w/v, aqueous) to bring the ash into solution.
- c) Pipette 100 > of each solution onto a 3/16 in. high purity National Carbon Products carbon electrode.
- d) Dry the electrodes, containing samples, under a heat lamp.

The emission spectrograph consisted of a National Spectrographic Laboratories power source and a large Hilger spectrograph with an interchangeable glass and quartz prism.

The spectrograph was operated on D.C. arc for greater sensitivity. Duplicate standards containing 0, 1, 5, 10, 50, and 100 μ g. of iron, respectively, were used to evaluate the extraction and spectrographic analysis procedures. The corrosion of stainless steel was measured by using diphenylcarbazide to determine the amount of chromium, the procedure being as follows: The procedures of Morrison and Freiser (1957), Urone and Anders (1950), and Sandell (1944) were modified in preparing the trisodium phosphate samples necessary. The following steps were involved:

- a) Clean all glassware by exposure to aqua regia for 15 minutes. Rinse the glassware seven times in tap water and three times in distilled water free from chromium contamination.
- b) Make chromium standards by placing 12 g. Na₂PO₄. 12H₂O and 25 ml. NaOCl in a 250 ml. Pyrex beaker containing approximately 150 ml. deionized distilled water. Add varying amounts of chromium standard solution (5 µg. Cr./ml., K₂Cr₂O₇, aqueous).
- c) Samples were obtained in an identical manner to those previously described. However, instead of transferring the evaporated 4-1. samples to a Berselius, tall-form 200 ml. beaker, transfer the samples into 250-ml., low-form Pyrex beakers.
- d) Acidify the standard and steel samples to pH 2 with conc. H_2SO_{μ} .
- e) Add 2 ml. 5% hydrazinedihydrochloride (w/v, aqueous), made daily, to each sample. Bring just to a boil.
- f) Bubble air, filtered through water, into the sample until a negative qualitative test for available chlorine is obtained. The test is done with starch iodide papers.
- g) Oxidize Cr to state VI by adding enough K2Mn04 crystals to give a residual pink upon bringing to a brief boil.
- h) Cool the samples to room temperature in a water bath containing tap water.

- i) Add a small amount of sodium azide (NaNz) crystals to reduce the residual K₂MnO4 color.
- j) If necessary, filter out any precipitates with asbestos supported by a coarse sintered glass filter.
- k) Adjust pH to exactly 1.9 with a Beckman (Model G) pH meter and 1:10 H₂SO₄ (v/v, aqueous).
- Add 1 ml. of 0.25% diphenylcarbazide solution (1:1 v/v, acetone and water) to each colorless sample. Allow 30 minutes for color development before taking the optical density (0.D.) reading.
- m) Quantitatively transfer samples to 200-ml. volumetric flasks and make to volume with deionized distilled water. Five hundred-ml. volumetric flasks may be substituted if color development is intense. Invert three times to mix.
- n) Check O.D. of all samples using a Beckman (Model B) spectrophotometer employing wavelength of 543 mu. and sensitivity of 3. The machine is adjusted to zero O.D. with the blank standard having no chromium.

Proprietary detergent samples were analyzed also for chromium. The procedures previously outlined for the trisodium phosphate samples were changed as described below:

- a) Make each chromium standard by placing 4 1. of tap water in a 4-1. Pyrex beaker. The tap water contains approximately 340 p.p.m. hardness expressed as CaCO₃ by the EDTA titration (Milk Industry Foundation, 1959). Add sufficient chromium standard solution (5 µg. Cr./ml., K₂Cr₂O₇, aqueous) volumetrically to give 0, 10, 50, and 100 µg. Cr per standard, respectively. Add 25 ml. of NaOCl to each 4-1. standard. Acidify each solution by adding 5 ml. conc. H₂SO₄ and enough conc. HNO₃ to give pH 2 to 3. Evaporate to approximately 150 ml. without boiling.
- b) Four-1. samples of chlorinated proprietary detergent were obtained from an automatic cleaning unit located in the Michigan State University dairy plant. Samples were taken after an elapsed

time of 30 minutes in the washing cycle. In this automatic cleaning unit, approximately 446 ft. of 1.5 in. stainless steel pipe were cleaned at a temperature of $138 \pm 3^{\circ}$ F. The available chlorine and detergent concentrations of the cleaning solutions varied widely. Available chlorine was furnished by NaOC1. The detergents were a mixture of Klenzade AC-1, and AC-6, used in approximately 2:1 ratio, respectively.

- c) Acidify the 4-1. samples, taken in 4-1. Pyrex beakers, by adding 5 ml. conc. H₂SO4, and enough conc. HNO3 to give a pH of 2 to 3. Evaporate to approximately 150 ml. without boiling.
- d) Wash the evaporated samples quantitatively into 250-ml. Pyrex beakers with distilled deionized water.
- e) Adjust the pH to exactly 1.9 with 30% NaOH (w/v, aqueous) and 1:10 H2SO4 (v/v, aqueous).
- f) Add a few drops of 5% hydrazinedihydrochloride (w/v, aqueous), made daily, to the warmed sample. Bubble air, filtered through water, into the sample until a negative qualitative test for available chlorine is obtained. The test is done with starch iodide papers.
- g) Add a large excess of KMnO4 crystals to the solutions and bring to a boil. Filter off the precipitate by filtering through asbestos supported by a coarse sintered glass filter. Repeat the KMnO4 oxidation and filtering twice more or until a residual KMnO4 color remains upon boiling at least one minute. Sintered glass filters are cleaned by aqua regia and detergent solutions, both of which are used in forward and reverse flow.
- h) Add a small amount of crystalline NaNz to reduce the residual KMnO4 color.
- i) Check, but do not adjust the pH of the sample solutions.
- j) Add 1 ml. of 0.25% diphenylcarbazide solution (1:1 v/v, acetone and water) to each colorless sample. Allow 30 minutes for color development before reading 0.D.

- k) Quantitatively transfer samples to 200-ml.
 volumetric flasks and make to volume with deionized distilled water. Five hundred-ml.
 volumetric flasks may be needed if color development is intense. Invert three times to mix.
- Check O.D. of all samples using a Beckman (Model B) spectrophotometer employing wavelength of 543 mu. and sensitivity of 3. The machine is adjusted to zero O.D. with the blank standard containing no chromium.

All statistical analyses were performed under the direction of W. D. Baten, Statistician, Michigan Agricultural Experiment Station. The term "significant" in this thesis refers to significance at the 1% probability level as calculated by the "t" test.

All reagents used were Reagent Grade unless otherwise noted.

EXPERIMENTAL RESULTS

Measurement of Soil Retaining Capacity

Methods of measuring films.

- a) Ellipsometer. No quantitative data for the method were tabulated. Films of barium stearate, 50 A. thick applied on number 4 and 7-finishes of stainless steel slides, were measurable within an accuracy of plus or minus 5 A. However, the thickness of films produced by pasteurized skimmilk on number 4-finish stainless steel slides and glass microscopic slides could not be measured with reliability, because of the roughness of the glass and stainless steel surfaces.
- b) Interferometer. The deflection of interference lines, caused by increasing the thickness of skimmilk films, is presented in the data of Table 3. Difficulty was encountered in attempting to evaluate thickness of skimmilk film with the interferometer because it was impossible to determine the number of deflections caused by one film of skimmilk. Deflections were noted visually to be equal approximately to one-half of a wavelength of sodium light per individual film layer. When the apparent deflection of interference lines was one-half, the observer had no way of knowing whether the deflection actually was one-half, one and one-half, or two and one-half, etc. For this reason, the interferometer

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method was discarded in this investigation.

- c) Electron microscope. The electron micrograph of a skimmilk film replica, taken at a magnification of 8,000 to 10,000, is shown in Figure 3. The large dark spots and their white shadows resulted from depositing droplets of dilute nitrocellulose on the glass surfaces prior to the skimmilk film. The droplet-shadows reflect film thickness. The variation in length of shadows of nitrocellulose droplets, having approximately the same diameter, supported the premise of film unevenness held by Rothen (1959). Shadows of equal length throughout the film would indicate uniform thickness. Perhaps the most significant point that might be noted from the micrograph is the rough surface topography revealed in the other areas of the micrograph not occupied by the nitrocellulose droplets and their shadows.
- d) Gravimetric. Comparisons of variation between the fourplace balance and another balance allowing estimation of the fifth place are illustrated in the data of Table 4.
 Variation was equal or greater with the five-place balance than with the four-place balance. Consequently, the latter was used throughout the study.

The uniformity of creating films of skimmilk on surfaces of glass and stainless steel, as measured gravimetrically, is illustrated in the data of Table 5. Evaluation Was made both within and between three individual racks

containing six slides each. Surfaces of stainless steel slides accepted weights of skimmilk film with approximately equal uniformity. A similar result was noted with glass slides. The data of Table 5 show a small variation in the weights of the film on the slides within and between racks relative to the filming procedure for one surface.

The increase in weight of 22 x 50 mm. micro cover glass slides after each application of skimmilk is shown in the data of Tables 6 and 7 and in Figures 4 and 5. After a slide was dipped once in skimmilk the weight of film deposited for each succeeding dip was linear, when the dipping was continued a maximum of 10 times.

Within one rack of glass slides the comparative weights of skimmilk films deposited per slide was quite uniform as shown in the data of Table 8. However, more variation in weight of film between the slides in one rack occurred when the slides were dipped 10 times, as compared to five.

Methods of evaluating cleaning.

a) Spectrophotelometer. The reproducibility of photelometer readings on 25 x 75 mm. glass microscope slides, dipped together in one rack, is given in the data of Table 9. These data show close agreement between repeated readings. When every alternate layer of film was made from a detergent mixture, instead of each

layer being skimmilk, there was also close agreement between three repeated readings (Table 10).

The reproducibility of photelometer values within and between three racks of microscope slides is again shown in the data of Table 11. Readings are given for clean slides, slides with films, and for slides with films after a controlled washing procedure. The readings for slides and films in racks 1 and 2 showed good agreement between and within racks. However, the readings made for slides in rack 3 showed wide variations. One possible reason was that the apparatus seemed subject to voltage variations. The effect of washing, determined by the difference between the percentage of light transmitted through the washed slide and the filmed glass slide, is given in the data of Table 12. High percentage light transmission values for the washed slides resulted, indicating almost complete removal of the film by the washing procedure.

Heating of skimmilk films decreased the percentage transmission of light as shown in the data of Table 13. A change in the crystalline structure of the film by heating could account for the difference in light transmission.

As has been noted, some difficulty was experienced in evaluating filming and washing procedures with the Spectrophotelometer. Nonreliability of the photelometer
method for the determination of quantitative filming is shown by data in Table 14 and illustrated in Figure 6. The absorbancy did not increase in a linear manner with increasing weight of film. That Beer's Law was not obeyed is confirmed by the data from Table 15 and Figure 7. The greatest departure from linearity is shown at both the higher and lower levels of film weight.

b) Microkjeldahl. Data in Table 16 show results from preliminary trials using the microkjeldahl method. The percentage recovery of the standard samples containing l mg. nitrogen, ranged from 89.5 to 101.1% with the mean recovery at 95%.

Wide differences in the comparative detergency values of hard and distilled water are illustrated in the data of Table 17. The soil film retained after washing, expressed in ug. of nitrogen per mg. of sample, showed a mean value of 15.1 for distilled water and 74.8 for the hard water.

The protein solubility of skimmilk-tap water films was increased significantly by chlorinating a 0.1% trisodium phosphate tap-water solution as shown in the data of Table 18. The protein remaining, expressed as age. of nitrogen per mg. sample, was decreased from 69.14 when no chlorine was used to 7.8 when 510 p.p.m. chlorine was employed. The increase in protein solubility was accompanied by an increase in pH of the solution from 9.4 to 10.4. The rise of pH could have been at least partially responsible for the increased protein removal. The pH of the trisodium phosphate tap-water solutions increased because sodium hypochlorite had been added for the purpose of chlorinating the solutions at different levels.

When buffer solutions at three different pH's were chlorinated, there was also a significant increase in protein solubility (data from Table 19, illustrated in Figure 8). In these trials a greater increase in protein solubility resulted for the nonchlorinated buffer than for the chlorinated buffer, causing an apparent disagreement with results of Table 18. Later results indicated that the pH of the buffers was not sufficiently high to show an increased protein removal by chlorination. Figure 8 also shows close correlation between the curve for the amount of 2-Molar sodium carbonate used in the buffer and the curve indicating protein solubility by the chlorinated buffer.

The effect of trisodium phosphate and sodium hypochlorite concentrations on the pH of tap water containing approximately 340 p.p.m. total hardness is shown in the data of Table 20. At a concentration of 0.5% trisodium phosphate the solution was practically stabilized at a pH of 11.4. A concentration of 0.15% trisodium phosphate in distilled water gave the same pH of 11.4. The addition of 12 ml. of sodium hypochlorite to both tap and distilledwater solutions at pH 11.4 increased the pH only 0.2. The data of Tables 22 and 23 show that significantly more protein was removed when 0.5% trisodium phosphate tapwater solutions and/or 0.15% trisodium phosphate distilledwater solutions were chlorinated. Both studies were performed at pH 11.5 \pm 0.1.

Numerous preliminary trials were performed to create a film sufficient in quantity and tenacity to permit measurements of cleaning for 10 minutes at 65° C. The information in Table 21 summarizes the procedures used in developing the tenacious films. Highest resistance to washing was secured with Film N, which was relatively high in protein and withstood unagitated washing for 10 minutes in a 0.15% trisdodium phosphate distilled-water solution. A variation of 36% in the protein content of these films was observed.

In preliminary studies time of reaction was important in obtaining increased protein solubility resulting from chlorination. The data of Table 24, Figure 9, confirmed the importance of the time factor. Protein solubility, due to chlorination, increased rapidly with time after one minute. At one-minute reaction time, no difference was found between the protein solubility of chlorinated and nonchlorinated solutions containing 0.15% trisodium phosphate in distilled water at pH 11.5 \pm 0.1. A bit of evidence that chlorination was not a detergent aid when used for short periods of time (less than 1 minute) was noted in making films. In preparing tenacious films,

slides were dipped momentarily and intermittently in clarifier slime suspensions and solutions of trisodium phosphate, calcium carbonate, and sodium hypochlorite. The build-up of such films was much more rapid than when the sodium hypochlorite was not used. The more rapid build-up was easily observed visually.

One of the objectives of the study was to determine optimum levels of chlorination. Data in Table 25, shown graphically in Figure 10, give the relationship of available chlorine to protein solubility under the conditions of this study. Increases in protein solubility were noted at all levels of chlorination studied. However, a more rapid increase in protein removal was noted above the 75 p.p.m. available chlorine level. A lower rate of increase was generally observed when the chlorine content exceeded 226 p.p.m. The data also show (Figure 10) that at the completion of washing, a smaller proportion of film remained on the slide above the 226 p.p.m. chlorine level than at the lower levels of chlorination. Therefore, probably insufficient film was present to permit the maximum protein removal possible by the use of available chlorine in the solution.

Methods of Evaluating Corrosion of Stainless Steel

<u>Conductivity</u>. Attempts to measure removal of metal by determining differences in conductivity of chlorinated distilled water solutions after exposure to type 304

stainless steel did not show positive results. No detectable difference in conductivity was found when 1 liter of agitated chlorinated distilled water was used to wash, collectively, five 25×75 mm. stainless steel slides. One problem was the small proportion of metal present, if any, in relation to the much larger proportion of detergent salts present.

Emission spectroscopy. Attempts to analyze used detergent samples for iron by the emission spectroscopy method were unsuccessful. Consequently, no quantitative data were collected.

Diphenylcarbazide for chromium VI. The diphenylcarbazide method for determining chromium VI was utilized to evaluate residues removed from type 304 stainless steel when treated with chlorinated trisodium phosphate and another proprietary chlorinated alkaline detergent. Data for the analyses of standard chromium samples containing trisodium phosphate and two different amounts of sodium hypochlorite are given in Table 26, and illustrated in Figure 11. Increasing the amount of sodium hypochlorite did not affect the chromium recovery from the standard sample. The location of the curve of Figure 11 was estimated visually. The curve was linear for the standard samples containing 0 to 120 µg. chromium.

As shown in the data of Figure 12, increasing the concentration of trisodium phosphate, in the standard samples, reduced the slope of the chromium recovery curve but did not affect its linear nature from 10 to 100 µg. chromium. However, the curve was not linear from 0 to 10 µg. chromium. In other words, the recovery of 0 to 10 µg. of chromium was not complete in the presence of 24 to 48 g. of trisodium phosphate. The curves were located visually as previously described.

When 1,120 sq. cm. of type 304 stainless steel were exposed to a chlorinated 0.3% trisodium phosphate distilled water solution, the amount of chromium removed is shown in the data of Table 27 and illustrated in Figure 13. Mean amounts of 1.5, 32.1, and 133 µg. chromium were removed from type 304 stainless steel when 0, 100, and 500 p.p.m. available chlorine, respectively, were used. Variation in chromium removed between individual samples was greater at the 100 p.p.m. chlorine level than at the 500 p.p.m. level. The mean rate of chromium removal increased with increasing chlorination but not necessarily in a linear manner (Figure 13).

Results of a preliminary trial showing the increase in chromium removal with the increase in temperature is presented in Figure 14. The curve appears to be linear in nature.

The data in Table 28 (illustrated in Figure 15) show the recovery of chromium from standard samples containing a proprietary chlorinated alkaline detergent of unknown composition. The data show that increasing the concentration of the detergent two-fold did not affect the chromium recovery. The curve was linear from 0 to 100 µg. chromium.

Samples employing the proprietary chlorinated alkaline detergent taken from a commercial automatic cleaning unit (Table 29) were negative in chromium, with the exception that a slightly positive test was secured from a detergent solution containing 234 p.p.m. available chlorine. The chromium content in the one positive sample was estimated to be 1 to 2 µg. in an original sample volume of 4 liters.

DISCUSSION

Perhaps the most significant result of this investigation was the development of a cleaning evaluation test based upon the microkjeldahl procedure. The procedure gave quantitative information regarding the functions of a detergent in removing protein at the soil-detergentsolution interface with results expressed in terms of nitrogen remaining on the soiled surface after the cleaning procedure. Maxcy and Shahani (1960) found that a somewhat similar procedure possessed sensitivity for 2 p.p.m. of skimmilk solids in testing detergent solutions after use.

There was a lack of uniformity in nitrogen recovery in preliminary trials of this investigation on "used" detergent solutions of chlorinated trisodium phosphate containing standard amounts of sample. An explanation for this phenomenon was offered by Cahn and Powell (1954) who reported that nitrogen was liberated when ammonia and hypochlorite were allowed to react in an alkaline medium. Therefore, nitrogen can be assumed to be lost to a variable degree during the washing procedure before the microkjeldahl analysis. On the basis of the report by Cahn and Powell, the results of examining used alkaline detergent solutions containing available chlorine for nitrogen content should be viewed with reservation. However, the value of the microkjeldahl method for determining nitrogen on

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soil films remaining after the washing procedure was well demonstrated in this investigation. Also, the absence of detergent chemicals in the kjeldahl flask facilitated the oxidation procedure by reducing the violence of the boiling.

Another significant result was the development of a special slime-type film high in protein and tenacity. Tenacious films were desirable in order to have sufficient film remain after the washing operation to furnish nitrogen for the relative comparisons of different conditions of cleaning. The films used by Roderig <u>et al</u>. (1956) were tenacious enough but too low in protein for effective comparisons of cleaning by the microkjeldahl method. The films employed by MacGregor <u>et al</u>., Claybaugh (1950), Jensen (1946), and others were not tenacious enough to withstand the rather rigorous washing conditions demanded in the present experiment.

Chlorination of trisodium phosphate, under conditions of this study, significantly increased protein solubility (removal) of soil films. Thus, one of the main objectives of the study, namely, the function of chlorination in detergency of alkaline cleaning materials, was partially explained.

The increased protein solubility of soil films due to chlorination of trisodium phosphate was dependent on time of exposure. After one minute the solubility of the

protein was much more rapid in the chlorinated than in the control solution. At one minute of exposure the protein solubility was identical for both the chlorinated and nonchlorinated solutions. An explanation for the poor detergency value of chlorinated trisodium phosphate at one minute or less of exposure was provided in the literature by many investigators who have noted that chlorine sanitizers combine with proteinaceous materials. Chlorine sanitizers are usually employed for short periods because of their fast action and corrosiveness to stainless steels.

The results of this investigation did not indicate clearly whether the relationship of beneficial protein solubility due to chlorination was linear with time of exposure to detergent. Further study was indicated. Another unclarified point was the importance of temperature on the beneficial effect of detergency due to chlorination. In these studies a beneficial cleaning effect was apparent at 65° C. but not at lower temperatures. There appeared to be almost a linear relationship between concentration of sodium hypochlorite and improvement in protein solubility. More investigation may be required to establish the effect of higher levels of chlorination. However, the higher levels of chlorination may contribute to corrosion problems.

A consideration of a combination of factors suggested that the colorimetric diphenylcarbazide method for measuring the amount of chromium VI was a promising technique

for evaluating corrosion of stainless steels. The postulation was based on measuring the amount of chromium in detergent solutions which had been exposed to stainless steel surfaces. Chromium was selected as the element for corrosion evaluation because it was present in stainless steels and when found in the used detergent solutions, it would most likely have been removed from the stainless steel surfaces. Unlike iron, chromium was not commonly present in water supplies. Also, metal removal from stainless steel was indicated to be in the order of small magnitude for any one exposure period to the detergent. The diphenylcarbazide method had been reported to be quantitatively sensitive to microgram quantities of chromium (Morrison and Freiser, 1957; and Saltzman, 1952). The surface composition of type 304 stainless steel used in this investigation was known (Rhodin, 1955).

In the studies reported herein, the diphenylcarbazide method for measuring chromium VI was demonstrated to be a useful and sensitive means of determining metal removal from stainless steel. To retain the distinct advantages of the test and to eliminate problems of analysis, future study should be made of a distillation procedure to effect a separation of chromium from the detergent samples. Chromyl chloride (CrO_2Cl_2) has been reported to be volatilized at 116° C. from mixtures of chromium, sulfuric acid and hypochlorites (Udy, 1956). The removal of



chromium from detergent samples by the formation of volatile chromyl chloride seems possible. If the chromyl chloride distillation was efficient in removing chromium, a diphenylcarbazide determination for chromium could be made on the distillate. Extreme sensitivity would then be attained without the interferences of detergent salts, heavy metals, organic matter, and hypochlorites. Success in developing the distillation procedure would make available a relatively simple, rapid method for evaluating removal of metal from stainless steels.

Many problems were involved in applying the diphenylcarbazide method for measuring chromium VI to the conditions of this investigation. The interferences have been mentioned above. The presence of the detergent salts made the samples very difficult to boil and resulted in losses of samples from "popping" in the later oxidation procedures. Another mechanical problem resulted from the method of taking samples. Four-1. samples of detergent were evaporated to approximately 150 ml. The probability of encountering measurable amounts of chromium was enhanced by taking the large samples, but the problems of any interferences present were magnified.

Oxidation to destroy organic matter by wet or dry ashing methods also created difficulties. Wet ashing with sulfuric acid, nitric acid, and perchloric acid, separately or in combination, resulted in loss of chromium by volatilization.

Dry ashing of the samples sometimes resulted in an ash which was insoluble or difficult to get into solution. Loss of samples through "popping" in the ashing procedure was also encountered.

Saltzman (1950) reported that phosphate was converted to polyphosphate by strong heating, either from wet ashing or dry ashing. Consequently, the chromium was complexed by the polyphosphate making the chromium nonreactive to diphenylcarbazide. Polyphosphates were undoubtedly present in this study and many of the negative results obtained were attributed to their presence.

Attempts to separate interferences with the chromium analysis by the troublesome extraction resulted in poor chromium recovery. As was true for the unsuccessful iron extractions, "sudsing" of the detergent and solvent was held responsible for the poor separations. Isoamyl alcohol, isoamyl alcohol and pyridine mixture, and chloroform were among the solvents used without success to extract the diphenylcarbazide-chromium complex.

Because of the difficulties encountered in separation methods and destruction of organic matter with wet and dry ashing, analysis for chromium was performed in aqueous solution. Potassium permanganate was employed to oxidize the organic matter present. This made possible the oxidation of chromium to the hexavalent state and permitted formation of the chromium-diphenylcarbazide complex.

In order that chlorites present did not interfere with the oxidation procedure, the hypochlorites were reduced to chlorides. Several reducing substances were tested before one was found which would reduce the hypochlorites to chlorides without interfering in the permanganate oxidation of chromium from a valence of three to six. Hydrazinedihydrochloride was the compound used successfully. Compounds used unsuccessfully were sodium azide, oxalic acid, hydroxylamine, stannous chloride, and hydrogen peroxide. Bubbling of air through the system assisted in eliminating any gaseous chlorine present.

The interference of heavy metals, primarily iron, was minimized by reading the diphenylcarbazide-chromium complex in 200 or 500 ml. volumes. The principle employed was to dilute the heavy metal interference (other than chromium) below that required for the diphenylcarbazide reaction and was in agreement with the report of Feigl (1946). Dilution of the samples was possible because of the extreme sensitivity of the diphenylcarbazide reaction for chromium.

The gravimetric procedure was superior to the other methods of film measurement used because of the relative uniformity of the film deposition, the reproducibility of the results obtained, and the flexibility of the method.

Comparison with the gravimetric method confirmed that the Spectrophotelometer method did not obey Beer's

Law for the conditions of this investigation. This is in accordance with the statements of Masurvosky and Jordan (1958) that light transmission measurements are deficient at both high and low concentrations of film.

The ellipsometer may have a very useful application in the measurement of stainless steel corrosion by detergents. The extreme sensitivity of the ellipsometer, as shown in these studies, supports this conclusion.

Though in an area outside the immediate scope of this study, the mechanical filming technique may have an application in measuring the bacteriological quality of milk. An illustration of its application is in the Breed smear procedure for bacterial enumeration. Skimmilk films formed by the mechanical technique were shown by the electron micrograph to be rough and uneven. On the other hand, milk films for the Breed smear are deposited and spread manually. Therefore, the Breed smears, presumably, would be less homogenous in bacterial content than the mechanically prepared film. If more attention was given to preparing the Breed smears, closer correlation between the Breed smear technique and other bacteriological enumeration methods might be expected.

SUMMARY AND CONCLUSIONS

Investigations were made of methods for measuring the function of chlorinated trisodium phosphate detergents. Methods were developed for evaluating a) the formation of milk films on glass and stainless steel surfaces, b) cleaning, and c) corrosion of stainless steel. These methods were applied under cleaning conditions of hard and distilled water, varied levels of chlorination, varied pH, and variations in time of exposure to the detergent.

The ellipsometer and other optical methods were ineffective in measuring thickness of skimmilk films because of the uneven nature of both the films and of the glass and stainless steel surfaces on which they were supported. Electron micrographs of skimmilk films supported this premise. Spectrophotelometer light transmission measurements of film deposition did not obey Beer's Law, particularly at both low and high levels of film thickness. Consequently, the method was considered unreliable for evaluation of cleaning.

Gravimetric measurements showed that the formation of skimmilk film on glass surfaces was a linear function when the slides were dipped from one to ten times.

A combination gravimetric and microkjeldahl method was effective in measuring the function of chlorinated trisodium phosphate detergents. Useful visual observations of cleaning were also available during the washing of the slides.

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Films of skimmilk on glass surfaces were not sufficiently resistant to washing with chlorinated trisodium phosphate to permit study after the cleaning procedure. Methods were developed for producing tenacious films from clarifier slime, calcium shloride, trisodium phosphate, and sodium hypochlorite.

Distilled water was much superior to hard water in aiding trisodium phosphate to remove skimmilk films from glass surfaces.

Improved detergency resulted from adding sodium hypochlorite to hard water. However, increased protein solubility was accompanied by an increase in pH. When the pH was stabilized at 11.5 ± 0.1 , and thus was unaffected by addition of sodium hypochlorite, the improved protein solubility was attributed to chlorination. Distilled water solutions of trisodium phosphate also showed improvement in cleaning ability after chlorination at pH 11.5 \pm 0.1.

At pH's lower than 11.5 ± 0.1 , chlorinated buffer solutions showed less ability to dissolve protein than did nonchlorinated buffers. However, ability of both chlorinated and nonchlorinated buffers to dissolve protein increased with an increase in pH.

Under the conditions of temperature and pH studied, the protein solubility due to chlorination of trisodium phosphate increased rapidly after one minute of exposure to the detergent.

An optimum level of chlorination was not found. Increased chlorination gave increased protein solubility at all levels of available chlorine used, 0 to 312 p.p.m. The least increase in rate of protein solubility occurred at 75 and 312 p.p.m. of available chlorine.

Chromium loss, of a small order of magnitude, occurred when type 304 stainless steel was exposed to chlorinated trisodium phosphate for 48 hours at 47° C. The chromium loss increased, as the available chlorine increased from 0 to 500 p.p.m., but not necessarily in a linear manner.

Chromium losses from a commercial system of stainless steel pipelines cleaned automatically with a proprietary chlorinated alkaline detergent were almost nonexistent. The highest available chlorine level of 234 p.p.m. yielded the only positive test and showed from 1 to 2 µg. of chromium present. All other tests were negative.

The following conclusions may be drawn from the investigation:

- a) Chlorination, by the addition of sodium hypochlorite, increases the capacity of trisodium phosphate solutions to remove milk-protein soils.
- b) Conditions contributing favorably to increased protein solubility are: a temperature of 65° C.;

a pH of 11.5 \pm 0.1; a time of exposure to the detergent of 10 minutes; and a high, though noncorrosive, available chlorine content.

- c) The capacity of detergent solutions to remove milk-protein soils can be successfully measured with a gravimetric-microkjeldahl technique.
- d) The metal removed from type 304 stainless steels by chlorinated trisodium phosphate solutions is of small magnitude. Such metal removal can be measured by testing the "used" detergent solution for chromium VI by the diphenylcarbazide method.

LITERATURE CITED

- Afanasev, B. W. (1948). The oxidation-reduction potential of chloramine and the mechanism of its oxidizing action. (In Russ.). Jour. Phys. Chem. (U.S.S.R.) <u>22</u>:499; (In Eng.). Chem. Abs. <u>42</u>:7169 g. (Original not seen).
- Albrecht, K. (1958). Diamond Alkali Co., Cleveland, Ohio. Personal correspondence, Oct. 22.
- Alexander, A. E. (1951). <u>Surface Chemistry</u>. Longmans, Green, and Co., New York. 70 pp.
- Ames, Ada M., and W. W. Smith. (1944). The temperature coefficient of the bactericidal action of chlorine. (Abs.) Jour. Bact. <u>47</u>:445.
- American Public Health Association, American Waterworks Association, and Federation of Sewage & Industrial Wastes Associations. (1955). <u>Standard Methods for</u> <u>the Examination of Water, Sewage, and Industrial</u> <u>Wastes</u>. 10th ed. Amer. Pub. Health Assoc., New York. 522 pp.
- Bacon, L. R., A. L. Sortier, and A. A. Roth. (1953). Field experience with Antibac, a new type of chlorine sanitizer. Food Technol. <u>16</u>:61-65.
- Barrett, R. B. (1958). Klenzade Products, Inc., Beloit, Wis. Personal correspondence, Oct. 13.
- Baskin, S. (1958). Time Chemical, Inc., Chicago, Ill. Personal correspondence, Oct. 14.
- Bass, S. L. (1960). Unpublished data. Mich. Agr. Expt. Sta., Agr. Chem. Dept.
- Bisio, A. L., H. B. Linford, and E. S. Saubestre. (1956). Cleanability and oil spreading reates. Indus. and Engin. Chem., News Ed. <u>48</u>:798-801.
- Blodgett, K. B. (1935). Films built by depositing successive monomolecular layers on a solid surface. Amer. Chem. Soc. Jour. <u>57</u>:1007-1022.

- Blodgett, K. B. (1937). Properties of built-up films of barium stearate. Jour. Phys. Chem. <u>41</u>:975-984.
- Botham, G. H. (1949). Corrosion of stainless steel by commercial sodium hypochlorite. 12th Internatl. Dairy Cong., Stockholm. <u>3</u>:310-317.
- Bothel, J. R. (1958). A. R. Maas Chemical Co., South Gate, Calif. Personal correspondence, Oct. 9.
- Braude, E. A., and F. C. Nachod. (1955). <u>Determination</u> of <u>Organic Structures</u> by <u>Physical Methods</u>. Academic Press, Inc., New York. 810 pp.
- Brookshier, R. K., and H. Freund. (1951). Separation of Chromium from vanadium. Analyt. Chem. <u>23</u>:1110-1113.
- Brunner, J. R. (1960). Unpublished data. Mich. Agr. Expt. Sta., Food Sci. Dept.
- Cahn, J. W., and R. E. Powell. (1954). The Raschig synthesis of hydrazine. Amer. Chem. Soc. Jour. <u>76</u>:2565-2567.
- Cahnman, H. J., and Ruth Bisen. (1952). Microdetermination of Chromium in blood. Analyt. Chem. <u>24</u>:1341-1345.
- Calbert, H. E. (1958). Influence of water temperature and hardness on cleaning of CIP farm milk pipelines. Jour. Milk and Food Technol. <u>21</u>(1):12-14.
- Carius, C. (1956). Potential forming processes on austenitic chrome-nickel steels in distilled water and in neutral halogen salt solutions. (In German). Arch. Eisenhütten 27:323-335; (In Eng.) Chem. Abs. 50:10632h. (Original not seen).
- Cazeneuve, P. (1900). Sur la diphenylcarbazide, reactif tres sensible de quelques composés metalliques: cuivre, mercure, fer au maximum, acid chromeque. Soc. Chim. de France Bul. Mem. 23:701-706.
- Clark, G., and G. G. Hawley. (1958). <u>The Encyclopedia of</u> <u>Chemistry</u>. Reinhold Pub. Co., New York. 1037 pp.
- Claybaugh, G. A. (1950). Investigations of detergency applicable to mechanical milk can washing. Thesis for degree of M.S., Mich. State Univ., East Lansing.
- Cohn, E. J. (1936). Influence of the dielectric constant on biochemical systems. Chem. Rev. <u>19</u>:241-273.

- Cox, G. A., and H. R. Whitehead. (1949). Chemical sterilization of dairy equipment. The bactericidal action of chlorine in the presence of protein. Jour. Dairy Res. <u>16</u>:327-333.
- Cucci, M. W. (1954). The use of radioactive phosphorus to measure the amounts of milkstone deposited on rubber, Pyrex glass, and Tygon tubings. Jour. Milk and Food Technol. 17:332-333.
- Davis, H. C., and A. Bacon. (1948). A study of some chemical reactions employed in photometric analysis. Soc. Chem. Indus. Jour. <u>67</u>:316-331.
- Davis, J. G. (1956). Cleaning and sterilizing in the dairy industry. (Rev.) Dairy Sci. Abs. <u>18</u>(7):527-553.
- Dean, R. B., O. Gatty, and E. Stenhagen. (1939). Disposition of protein multilayers. Nature. [London]. <u>143</u>:721-722.
- Faber, H. A. (1947). Contemporary chlorination practices. Amer. Waterworks Assoc. Jour. <u>39</u>:200-208.
- Fair, G. M., J. C. Morris, S. L. Chang, I. Weil, and R. P. Burden. (1948). The behavior of chlorine as a water disinfectant. Amer. Waterworks Assoc. Jour. <u>40</u>:1051-1061.
- Feigl, F. (1946). <u>Qualitative Analysis by Spot Tests</u>. 3d ed. Elsevier Pub. Co., Inc., New York. 574 pp.
- Finley, R. D., and M. J. Foter. (1947). A study of the corrosion of tin plate by can washing compounds. Jour. Milk and Food Technol. <u>10</u>:263-268.
- Fleischman, F. F., Jr., and R. F. Holland. (1953). The cleaning of glass piping in dairy plants. Jour. Milk and Food Technol. <u>16</u>:9-14.
- Fluette, E. R. (1960). The Diversey Corp., Chicago, Ill. Unpublished data and personal correspondence, Apr. 12.
- Foster, E. M. (1949). Quaternaries vs. chlorine in bacteria control. Jour. Milk and Food Technol. <u>12</u>:13-18.
- Gilcreas, F. W., and J. E. O'Brien. (1941). Laboratory studies of methods for cleaning of eating utensils and evaluating detergents. Amer. Jour. Pub. Health. 13:143-150.

- Greenland, K. M. (1952). Measurement and control of the thickness of thin films. Vacuum <u>2</u>:216-230.
- Gunn, A. F., and R. A. Scott. (1946). Measurement of thickness of thin films. Nature. [London]. 158:621.
- Haller, H. S., F. M. Grant, and C. J. Babcock. (1941). The corrosive effect of chlorine and lye solutions on metals used in dairy equipment. U.S. Dept. Agr. Tech. Bul. 756. 27 pp.
- Harding, H. G., and H. A. Trebler. (1947). Detergents for dairy plants and methods for their evaluation. Food Technol. <u>1</u>:478-493.
- Harper, W. J. (1958). A radiometric method for measuring stainless steel corrosion. (Mimeo) Paper presented, 53rd Ann. Meeting, Amer. Dairy Sci. Assoc. 5 pp.
- Hays, G. L., J. D. Burroughs, and D. H. Johns. (1958). The cleanability of materials in contact with dairy products. Jour. Milk and Food Technol. 21:68-72.
- Hays, G. L., J. D. Burroughs, and L. R. Pearson. (1960). The cleanability of materials in contact with dairy products. II. The cleanability of methyl methacrylates. Jour. Milk and Food Technol. 23:19-23.
- Herken, H., and H. O. Silbersiepe. (1951). Hydrolysis of peptide bonds of protein by hypochlorite. (In German). Arch. Expt. Path. and Pharmakol. <u>212</u>:205-213; (In Eng.). Chem. Abs. <u>47</u>:11270^b. (Original not seen).
- Herriott, R. M. (1947). Reactions of native proteins with chemical reagents. pp. 169-225, in M. L. Anson and J. T. Edsall, ed. <u>Advances in Protein Chemistry</u>. III. Academic Press, Inc., New York. 524 pp.
- Hiscox, E. R., and C. A. E. Briggs. (1955). Reviews of the Progress of dairy science. B. Bacteriology and mycology applied to dairying. Jour. Dairy Res. 22:391-413.
- Holland, R. F., J. D. Shaul, D. A. Theokas, and H. M. Windlan. (1953). Cleaning stainless steel lines in place. Food Engin. 25(5):75-78.

- Hucker, G. J., A. J. Emery, and Elizabeth Winkler. (1951). The relation of soil film build-up and low surface wetting properties to plastic and china surfaces. Jour. Milk and Food Technol. <u>14</u>:95-97.
- Hucker, G. J. (1954). The adherence of organisms and soil to surfaces of eating utensils. Jour. Milk and Food Technol. <u>17</u>:48-51.
- Hughes, R. C., and R. Berstein. (1945). Machine dishwashing compounds. Indus. and Engin. Chem., News Ed. 37:170-175.
- Jacobsen, D. H. (1954). How to care for your stainless steel equipment. Jour. Milk and Food Technol. <u>17</u>:55-57.
- Jennings, W. G. (1959a). Circulation cleaning. II. Effects of entrained air. Jour. Dairy Sci. <u>42</u>:476-479.
 - . (1959b). Circulation cleaning. III. The kinetics of a simple detergent system. Jour. Dairy Sci. <u>42</u>:1763-1771.
- Jennings, W. G. (1961). A critical evaluation of <u>in vitro</u> radioactive phosphorus additions for estimating soil deposits. Jour. Dairy Sci. <u>44</u>:258-268.
- Jennings, W. G., A. A. McKillop, and J. R. Luick. (1957). Circulation Cleaning. Jour. Dairy Sci. <u>40</u>:1471-1479.
- Jensen, J. M. (1946). Measuring detergency functions as affected by various detergents and procedures against milk films by application of a mechanical washing apparatus. Jour. Dairy Sci. <u>29</u>:453-463.
- Jensen, J. M., L. G. Harmon, and T. I. Hedrick. (1959). Recirculation-spray versus manual cleaning of milk storage tanks. Jour. Milk and Food Technol. <u>22</u>:206-208.
- Jensen, J. M., and J. Waterson. (1950). A method for measuring the cleanliness of milk cans. Jour. Milk and Food Technol. <u>13</u>:332-335.
- Johns, C. K. (1951a). Application of chemical germicides. Milk Plant Monthly. <u>40(9):72-74</u>.

. (1951b). The germicidal effectiveness of a new chlorine compound. Jour. Milk and Food Technol. <u>14</u>:134-136.

- Johnson, J. J., and C. T. Roland. (1940). Study of dairy cleaning problems. I. Films and deposits on hotmilk equipment. Jour. Dairy Sci. 23:457-461.
- Joly, M. (1939). Sur l'existence de deux formes de couches superficielles de protéines. Compt. Rendt. 208:975-976.
- Kaufmann, O. W., R. H. Andrews, and P. H. Tracy. (1955). Further studies on in-place cleaning. Jour. Dairy Sci. 38:371-379.
- Kaufmann, O. W., T. I. Hedrick, I. J. Pflug, C. G. Pheil, and R. A. Keppler. (1960). Relative cleanability of various stainless steel finishes after soiling with inoculated milk solids. Jour. Dairy Sci. 43:28-41.
- Kaufmann, O. W., and P. H. Tracy. (1959). Formation and removal of an iridescent discoloration in cleanedin-place pipelines. Jour. Dairy Sci. <u>42</u>:1883-1885.
- Klenzade Products, Inc. (1960). <u>Klenzade Dairy Sanita-</u> <u>tion Handbook</u>. 4th ed. Klenzade Products, Inc., Beloit, Wis. 212 pp.
- Langmuir, I. (1938). Properties and structures of protein films. Proc. Rog. Inst. Gt. Brit. Advance copy Dec. 9. 14 pp.; Chem. Abst. <u>33</u>:4612¹. (Original not seen).
- Langmuir, I., D. J. Schaeffer, and D. M. Wrinch. (1937). Built up films of proteins and their properties. Science. <u>85</u>:76-80.
- Lasmanus, J., and G. R. Spencer. (1953). The action of hypochlorite and other disinfectants on Micrococci with and without milk. Amer. Jour. Vet. Res. <u>14</u>:514-516.
- Leeder, J. G. (1956). Milkstone it can be controlled. Jour. Dairy Sci. <u>39</u>:229-230.
- Leeder, J. G., and R. V. Martin. (1952). Effects of the Hydrotron on milk and milkstone formation. Jour. Dairy Sci. <u>35</u>:920-928.



- Leenerts, L. O., J. F. Pietz, and J. Elliott. (1956). Soil removal by dishwashing detergents. Jour. Amer. Oil Chem. Soc. <u>33</u>:119-122.
- Lehn, G. J. (1946). Surface chemistry in chemical cleaners. Milk Plant Monthly. <u>35</u>(7):50-53.
- Leighty, M. (1958). Bonewitz Chemicals, Inc., Burlington, Iowa. Personal correspondence, Oct. 9.
- Lewandowski, T. (1954). Laboratory study of milkstone formation. (Abs.) Paper presented, 49th Ann. Meeting, Amer. Dairy Sci. Assoc.; Jour. Dairy Sci. 37:640.
- Lewandowski, T. (1959). CIP cleaners. (Mimeo) Proc. 28th Ann. Meeting, Wash. State Col. Instit. Dairying, Pullman, Wash. 5 pp.
- Lindquist, B. (1953). Some aspects on wetting agents for use in bottle washing compounds. 13th Internatl. Dairy Cong., The Hague. <u>3</u>:877-882.
- Little, L. L. (1947). Review of the literature pertaining to the chemistry of can washing. Milk Plant Monthly <u>36</u>:22-25.
- Lucy, F. A. (1948). Studies of surface films by reflection of polarized light. Jour. Chem. Phys. <u>16</u>:167-174.
- Luecke, R. W. (1960). Unpublished data. Mich. Agr. Expt. Sta., Agr. Chem. Dept.
- Lyness, W. I., and F. W. Quackenbush. (1955). Jour. Amer. Oil Chem. Soc. <u>32</u>:520-521.
- MacGregor, D. R., P. R. Elliker, and G. A. Richardson. (1954). Effect of added hypochlorite on detergent activity of alkaline solutions in recirculation cleaning. Jour. Milk and Food Technol. <u>17</u>:136-138.
- Masurvosky, E. B., and W. K. Jordan. (1958). Studies on the relative cleanability of milk contact surfaces. Jour. Dairy Sci. <u>41</u>:1342-1358.
- Masurvosky, E. B., and W. K. Jordan. (1960). Studies on the removal of <u>Staphylococcus aureus</u> from milkcontact surfaces by ultrasonic cleaning methods. Jour. Dairy Sci. <u>43</u>:1545-1559.
- Maxcy, R. B. (1958). The Diversey Corp., Chicago, Ill. Personal correspondence, Oct. 9.

- Maxcy, R. B., and K. M. Shahani. (1960). Evaluation of circulation cleaning of welded pipelines. (Abs.) Paper presented, 55th Ann. Meeting, Amer. Dairy Sci. Assoc.; Jour. Dairy Sci. <u>43</u>:856-857.
- McMeeken, T. L., and B. D. Polis. (1949). Milk Proteins. pp. 201-228, in M. L. Anson and J. T. Edsall, ed. <u>Advances in Protein Chemistry</u>. V. Academic Press, New York, 481 pp.
- Mead, M., and J. V. Pascoe. (1952). A method for assessing the cleaning efficiency of detergents. Austral., Jour. Dairy Technol. 7:114; Jour. Dairy Res. 22:407-408. (Original not seen).
- Meewes, K. H. (1951). Die mikrobiologische Prüfung von Desinfektionsmitteln unter Bedingungen der Molkerei-Fraxis. Kieler Milchw. Forsch. Ber. 3(3):251-258; Dairy Sci. Abs. <u>14</u>:938. (Original not seen).
- Mellanby, J. (1906). Globulin. Jour. Physiol. 8:527-528.
- Milk Industry Foundation. (1959). Laboratory Manual. 3d ed. Milk Industry Found., Washington. 838 pp.
- Milk Industry Foundation. (1957). <u>Manual for Milk Plant</u> <u>Operators</u>. 2d ed. Milk Industry Found., Washington. 635 pp.
- Mitten, H. L., Jr. (1954). Corrosion of dairy metals. Amer. Milk Rev. <u>16</u>(2):50,52,54,56,118.
- Mohr, W., and R. Junger. (1953). Die Prüfung und Beurteilung von Reinigungsmitteln. 13th Internatl. Dairy Cong., The Hague. <u>3</u>:851-857.
- Mohr, W., R. Junger, and E. Weinert. (1953). Reinigung von Milchflaschen. 13th Internatl. Dairy Cong., The Hague. <u>15</u>:886-871.
- Mohr, W., and E. Mohr. (1954). Adhäsion (Benetzung) von Reinigungsmittellösungen gegenüber verschiedenen Materialen (vorläufige Mitteilung). Kieler Milchw. Forsch. Ber. <u>6</u>(1):29-39; Dairy Sci. Abs. <u>17</u>:304. (Original not seen).
- Mohr, W., E. Weinert, and K. H. Peters. (1953). Prüfung von Reinigungsmitteln für die Reinigung von Milchkannen und Milchwirtschaftlichen Geräten mit Bürsten von Hand. Kieler Milchw. Forsch. Ber. 5(3):261-272; Dairy Sci. Abs. 17:304-305. (Original not seen).

- Mohr, W., and A. Nortmann. (1953). Prüfung von Reinigungsmitteln in Korrosionversuch gegenüber Materialien der milchwirtschaftlichen Apparate und Geräte (Aufstellung einer Normalversuchsordnung). Milchw. 8(4):111-118; Dairy Sci. Abs. <u>16</u>:337-378.
- Mohr, W., A. Wortmann, and H. Gerauer. (1952). Bestimmung der Alkalitat in Reinigungsmittellosungen. Kieler Milchw. Forsch. Ber. <u>4</u>(4):421-445; Dairy Sci. Abs. <u>15</u>:995-996. (Original not seen).
- Morrison, G. H., and H. Freiser. (1957). <u>Solvent Extrac-</u> <u>tion in Analytical Chemistry</u>. John Wiley & Sons, Inc., New York, 269 pp.
- Moulin, A. (1904). Dosage colorimetrique du chrome. Soc. Chim. de France Bul. Mem. <u>31</u>:295-296.
- Myrick, N. (1954). Stainless steel. Amer. Milk Rev. <u>16</u>(10):72,74,78,144-147.
- Niven, W. W., Jr. (1955). <u>Industrial Detergency</u>. Reinhold Pub. Co., New York. 331 pp.
- Overman, O. (1959). What is new in cleaners and sanitizers. (Mimeo) Paper presented 7th Ann. Dairy Mfgrs. Conf., Univ. of Ky., Lexington. 7 pp.
- Feters, J. J., and H. E. Calbert. (1960). Use of P³² in the study of the cleanability of milk handling surfaces. (Abs.) Paper presented, 54th Ann. Meeting, Amer. Dairy Sci. Assoc.; Jour. Dairy Sci. <u>43</u>:857.
- Ramsdell, G. A., and E. O. Whittier. (1953). The effect of heat on the albumin and globulin in milk. Jour. Dairy Sci. <u>36</u>:437-439.
- Reddish, G. F. (1957). <u>Antiseptics</u>, <u>Disinfectants</u>, <u>Fungi-</u> <u>cides</u>, <u>and Sterilization</u>. 2d. ed. Lea & Febiger. <u>Philadelphia</u>. 975 pp.
- Ressler, G. (1958). Blockson Chemical Co., Joliet, Ill. Personal correspondence, Oct. 16.
- Rhodin, T. N. (1955). Chemical analysis of thin films by x-ray emission spectrography. Analyt. Chem. 27:1857-1861.
- Ridenour, G. M., and E. H. Armbruster. (1953). Bacterial cleanability of various types of eating surfaces. Amer. Jour. Pub. Health. <u>43</u>:138-149.

- Roderig, H., L. F. L. Clegg, H. R. Chapman, J. A. F. Rook, and W. A. Hoy. (1956). Jour. Soc. Dairy Technol. <u>9</u>:75-80; Chem. Abs. <u>50</u>:14136i.
- Roland, C. T. (1942). Application of the newer phosphates in dairy operations. Milk Plant Monthly. <u>31</u>(6):38, 45,46.
- Rothen, A. (1956). Surface film techniques. pp. 155-199, in G. Oster and A. W. Pollister, ed. <u>Physical</u> <u>Techniques in Biological Research</u>. II. Academic Press, Inc., New York. 502 pp.
- Rothen, A. (1959). The Rockefeller Institute, New York, N. Y. Personal correspondence, Sept. 30.
- Rothen, A., and Marjorie Hanson. (1948). Optical measurements of surface films. I. Rev. Sci. Instruments. 19:839-841.
- Rowland, G. P. (1939). An optical study of permanganate ion and of the chromium-dyphenylcarbazide system. Indus. and Engin. Chem., Analyt. Ed. <u>11</u>:442-445.
- Rupple, J. G. (1960). Detergent requirements for various types of food soils. (Mimeo) Paper presented, 22nd Ed. Seminar, Klenzade Products, Inc., Beloit, Wis. 2 pp.
- Saltzman, B. F. (1952). Microdetermination of chromium with diphenylcarbazide by permanganate oxidation. Analyt. Chem. <u>24</u>:1016-1020.
- Sandell, E. B. (1944). <u>Colorimetric Determination of</u> <u>Traces of Metals</u>. III. Interscience Pub., Inc., New York. 487 pp.
- Scales, F. M., and Muriel Kemp. (1940). Application of wetter water to dairy and milk plant use. Jour. Milk Technol. <u>3</u>:221-227.
- Schwartz, C. (1941). Detergents in the dairy industry. Jour. Milk Technol. <u>4</u>:258-267.
- Seiberling, D. A., and W. J. Harper. (1956). Evaluation of the cleanability of CIP automatic valves. (Abs.) Paper presented, 51st. Ann. Meeting, Amer. Dairy Sci. Assoc.; Jour. Dairy Sci. <u>39</u>:919-920.
- Shepard, M. E. (1960). Chemical application and control devices. (Mimeo) Paper presented, 22nd Ed. seminar, Klenzade Products, Inc., Beloit, Wis. 5 pp.

- Shere, L. (1942). How to prevent and remove milk deposits. Food Indus. <u>14</u>(6):44-46.
- Shogren, C. B. (1951). Modern detergents as an aid to improved sanitation procedures. Amer. Milk Rev. <u>13</u>(9):44-46,48,50,52,84.
- Sisley, J. P., and P. J. Nood. (1952). <u>Encyclopedia of</u> <u>Surface</u> <u>Active</u> <u>Agents</u>. Chem. Pub. Co., New York. 540 pp.
- Sommer, H. H. (1938). <u>Market Milk and Related Products</u>. H. H. Sommer, Madison, Wis. 699 pp.
- Trebler, H. A. (1945). Newer dairy cleaners. Milk Plant Monthly <u>34</u>(12):47-48.
- Trurnit, H. J. (1959). Rias, Inc., Baltimore, Md. Personal correspondence, May 5.
- Udy, M. J. (1956). <u>Chromium</u>. I. <u>Chemistry of Chromium</u> <u>and Its Compounds</u>. Reinhold Pub. Co., New York. 433 pp.
- Uhlig, H. H. (1948). <u>The Corrosion Handbook</u>. John Wiley & Sons, Inc., New York. 1188 pp.
- Urone, P. F. (1955). Stability of colorimetric reagent for chromium, s-diphenylcarbazide, in various solvents. Analyt. Chem. 27:1354-1355.
- Urone, P. F., and H. K. Anders. (1950). Determination of small amounts of chromium in human blood, tissues, and urine. Analyt. Chem. 22:1317-1321.
- Van Hall, C. E. (1954). 4 Aminopyridine as a standard in acidemitry. Thesis for degree of M.S., Mich. State Univ., East Lansing.
- Weber, G. R., R. Bender, and M. Levine. (1940). Effect of ammonia on the germicidal efficiency of chlorine in neutral solution. Jour. Bact. <u>40</u>:145.
- Welcher, F. J. (1947). <u>Organic Analytical Reagents</u>. III. D. Van Nostrand Co., New York, 593 pp.
- Wildbrett, G., J. Gay, and F. Kiermeier. (1954). Uber die Prüfung der reinigenden Wirkung von Reinigungsmitteln für Handarbeit. Milchwissenschaft <u>9</u>(12): 410-414; Dairy Sci. Abs. <u>17</u>:751.

- Wilkowske, H. H., and W. A. Krienke. (1955). Inhibitory substances in milk. Jour. Milk and Food Technol. <u>18</u>:254-261.
- Wilson, H. N., and J. G. M. Bremner. (1948). Disproportionation in inorganic compounds. Jour. Chem. Soc. London. Quart. Rev. II. <u>1</u>:1-24.
- Wright, H. E. (1958). DuBois Co., Inc., Cincinnati, Ohio. Personal correspondence, Oct. 24.
- Wright, N. C. (1926). The action of hypochlorites on amino acids and proteins. Biochem: Jour. <u>20</u>:524-532.
- Wright, N. C. (1936). The action of hypochlorites on amino acids and proteins. The effect of acidity and alkalinity. Biochem. Journ. <u>30</u>:1661-1667.
- Yearian, H., W. D. Derbyshire, and J. F. Radovich. (1957). The formation of oxide films on chromium and 18 Cr-8 Ni steels. Corrosion <u>13</u>:597t-607t; Chem. Abs. 51:16246g. (Original not seen).
- Young, J. W. (1958). Tykor Products, Chicago, Ill. Personal correspondence, Oct. 14.
- Yuen, H. B. (1958). Pfanstiehl Detergent Chemicals, Inc., Waukegan, Ill. Personal correspondence, Oct. 16.
- Zaelke, A. E. (1958). Turco Products, Inc., Los Angeles, Calif. Personal correspondence, Oct. 16.
- Zoller, F. H. (1923). The rate of decomposition of sodium hypochlorite in cow's milk. Jour. Dairy Sci. <u>6:310-319</u>.

Slide	Times dipped in skimmilk (no.)	Deflection* (no.)
A	1	が
B	2	0
C	3	が
D	4	0
E	5	が
F	6	0

TABLE	3.	-	Skimmilk	film	depos	sition	on	glass	microscope
	slides measured by interferometer						_		

* Deflections in wave lengths of sodium light.

C144	Variation			
SIIde	4th decimal* (mg.)	5th decimal* (mg.)		
A B C D E F	0.1 0.0 0.0 0.0 0.1 0.1	0.18 0.02 0.00 0.03 0.07 0.09		
G H J K L	0.0 0.0 0.0 0.0 0.0	0.08 0.06 0.03 0.01 0.04 0.09		
M N O P Q R	0.4 0.1 0.1 0.0 0.1 0.1	0.01 0.04 0.35 0.06 0.07 0.05		
Total	1.1	1.28		
x	0.06	0.07		

TABLE 4.	-	Variation in weight of slides when weighed t	;0
		the 4th and to the 5th decimal place	

* Represents mg. variation of three repetitive weighings.
| | Film | weight | of slide | s dipped | in skim | milk |
|--------------------|----------------------------|--|-----------------------|--|----------------------------|--|
| Slide
Surface | Rack 1 | | Rack 2 | | Rack 3 | |
| | Slide | Film
weight
(mg.) | Slide | Film
weight
(mg.) | Slide | Film
weight
(mg.) |
| Stainless
steel | A
B
C
D
E
F | 2.7
2.8
3.1
2.4
2.7
3.2 | G
H
J
K
L | 3.2
2.7
2.7
3.0
2.8
2.6 | M N O P Q R | 3.2
3.0
2.9
2.8
2.8
3.0 |
| Ī | | 2.8 | | 2.8 | | 2.9 |
| Glass | A
B
C
D
E
F | 3.3
3.8
3.6
3.5
3.5
3.4 | G
H
J
K
L | 3.4
3.5
3.3
3.5
3.7
3.7 | M
N
O
P
Q
R | 3.4
3.4
3.4
3.7
3.5
3.5 |
| x | | 3.5 | | 3.5 | | 3.4 |

TABLE 5. - Uniformity of skimmilk film weights on 25 x 75 mm. slide surfaces measured gravimetrically

Film	Times dipped (no.)	Film weight* (mg.)
A B C D E	1 2 3 4 5	2.1 4.2 6.0 9.7
F G H J	6 7 8 9 10	11.3 12.4 16.0 18.7

TABLE 6. - Skimmilk film formation on 22 x 50 mm. micro cover glass slides when dipped intermittently and measured gravimetrically

* Represents mean of two trials.

TABLE 7. - Skimmilk film formation on 22 x 50 mm. micro cover glass slides measured gravimetrically

Film	Times dipped (no.)	Film weight* (mg.)	
A	1	2.5	_
B	2	4.1	
C	3	5.1	
D	4	6.6	
E	5	7.8	

* Represents mean of two trials.



5	dips	10	dips
Slides*	Film weight (mg.)	Slides*	Film weight (mg.)
A B C D E	5.9 6.1 6.1 6.3 6.6	A B C D E	17.8 21.4 25.0 25.1 24.9
F G H J	5.9 6.2 6.1 6.2 6.1	F G H I J	23.1 20.6 19.6 25.6 20.3
Variation	0.7	Variation	7.8

TABLE 8. - Variation in skimmilk film weight per rack of 22 x 50 mm. micro cover glass slides as affected by number of dips

* Represents slides dipped collectively in one rack.

Clida	Percentage	transmittancy	
SIIde	lst reading* (%)	2nd reading* (%)	
A B C D E F	92 91 92 91 93 93	91 91 92 91 92 93	
G H J K	93 93 91 92 93	93 93 91 93 93	

TABLE	9.	-	Reproducibility of Spectrophotelometer readings
			on 25 x 75 mm. glass microscope slides filmed
			with skimmilk

* Represents mean of three respective slide positions.

();;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;	Percentage transmittancy			
SILLES	lst reading*	2nd reading*	3rd reading*	
	(%T)	(%T)	(%T)	
A	81	8 3	85	
B	81	84	84	
C	80	83	84	
D	80	83	83	
E	81	84	84	
F	80	82	83	
G	80	81	83	
H	79	80	82	
I	79	79	82	
J	79	75	81	

TABLE 10. - Reproducibility of Spectrophotelometer readings on 25 x 75 mm. microscope slides filmed intermittently with skimmilk and a detergent mixture

* Represents slides dipped collectively in one rack, 4 dips each in detergent mixture and in skimmilk.

* Represents mean of three respective slide positions.

75 Spe	mm. glass sctrophote	microscop lometrical	e slides as .ly 	measured
Slide		Percentage	e transmitta	ncy
(no.)	Clean slide (%T)	Filmed slide* (%T)	Cleaned slide* (%T)	Cleaning loss (%T)
lst rack 1 2 3 4 5 6 7 8 9 10 11	100 100 100 100 100 100 100 100 100 100	80 81 83 81 86 86 83 83 83 83 83 83	91 92 93 91 93 94 93 92 93 92 91	11 11 10 10 7 8 10 9 10 9
2nd rack 12 13 14 15 16 17 18 19 20 21 22	99 100 100 100 99 98 100 100 99 98 99	83 84 83 85 85 82 81 81 81 81 80 82	92 93 93 92 93 91 91 91 88 89 92	9 9 9 8 7 9 10 10 7 9 10
3rd rack 23 24 25 26 27 28 29 30 31 32	99 98 100 99 98 100 100 97 98	80 81 82 83 85 83 81 78 76 75	89 89 91 91 92 90 86 86	9 8 9 8 7 7 10 12 10 11

TABLE 11. - Reproducibility of filming and cleaning procedure within and between three racks of 25 x 75 mm. glass microscope slides as measured Spectrophotelometrically

Slide	Percentage	e transmittancy
SIIde	Filmed slide* (%T)	Washed slide* (%T)
lst rack A B C D E F	84 85 85 85 85 85	99 98 99 97 98 97
2nd rack G H I J K L	78 76 77 76 76 80	98 97 98 98 98 98
3rd rack M N O P Q R	80 82 84 81 81 80	97 97 98 98 98 98

TABLE	12.	 Removal of skimmilk films from 25 x 75 mm.
		glass microscope slides by controlled washing
		as measured Spectrophotelometrically

* Represents mean of three respective slide positions.

Slide	Perc	centage transm	ittancy
DIIde	Unheated 1 (%T)*	film Heat	ed film ⁺ (%T)*
lst rac	k		20
A B	92		89
č	93		89
	•		
2nd rac D	K 92		89
Ĕ	93		89
F	94		91
3rd rac	k		
G	- 93		89
H	93	93 89	
TABLE 14 S d s	pectrophotelometer ips of 25 x 75 mm. lides into skimmil	r absorbancy v glass micros .k	s. number of
	Miner dinned	Absor	bancy
L T T W	(no.)	Trial 1 (Ag)	Trial 2 (A _g)
A	1	0.061	0.068
B	2	0.058	0.072
D	2 4	0.076	0.118
E	5	0.127	0.150
F	6	0.146	0.127

TABLE 13.	-	Effect of heating on Spectrophotelometer read-
		ings of skimmilk filmed 25 x 75 mm. glass
		microscope slides

Ŧ	llm	Times dipped (no.)	Filı	n weight (mg.)	Absorbancy
	A B C D E F	1 2 3 4 5 6		3.1 3.8 4.1 6.0 7.2 8.4	0.068 0.072 0.078 0.118 0.150 0.127
TABLE	16	Recovery of 1 m samples of (NH4 Kjeldahl method	es. of)2804	nitrogen as measu	from standard red by the micro-
		Standard (no.)		Recov ()	very %)
		1 2 3		93 89 94	.4 •5 •8
		4 5 6		95 95 101	.4 .8 .1
			$\overline{\mathbf{x}}$	95	.0

TABLE 15. - Sensitivity of gravimetric vs. Spectrophotelometer methods of measuring skimmilk film on 25 x 75 mm. glass microscope slides

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meri - 1	Film remaining	; after washing
Triai	Distilled water (µg. N/mg. sample)*	Hard water ⁺ (µg. N/mg. sample)*
A B C D	15.8 17.4 15.1 12.1	71.6 72.2 73.0 76.6
x	15.1	74.8

TABLE 17. - Effect of water hardness on detergency of 0.1% trisodium phosphate solutions in removing skimmilk films as measured by micro-Kjeldahl procedure

+ Represents 340 p.p.m. total hardness.

* Represents μg . nitrogen remaining after washing per mg. film sample (3 min. unagitated wash at 45° C.).

TABLE 18. - Protein solubility of skimmilk-tap water soil film as influenced by chlorination of 0.1% trisodium phosphate solutions in tap water

Sodium hypochlorite (ml.)	Available chlorine (p.p.m.)	ЪĦ	Residual film* (µg. N/mg. sample)	đ
0.0	0	9.4	69.14	2.59
2.0	92	9.7	50.85	5.52
4.3	191	10.0	30.32	8.24
6.6	283	10.0	24.71	12.80
12.0	510	10.4	7.80	5.74

* Represents mean of 6 trials, μg . nitrogen removed by washing per mg. film sample (10 min. unagitated wash, 65 to 54° C.).

					_	
	Unchlorins buffer	ited	Chlorinat buffer	ed	- Buf compo	fer sition
(pH)	Residual film* (µg. N/mg. sample)	6	Residual film+ (ug. N/mg. sample)	б	2M. NaCO3 (ml.)	2M. NaHCO3 (ml.)
9.4	74.70	1.43	81.69	2.29	47.5	202.5
10.0	23.12	1.56	42.01	9.75	137.5	112.5
10.7	7.08	0.29	10.19	1.49	225.0	25.0

TABLE 19. - Effect of chlorinated buffer solutions at different pH's on protein solubility of skimmilktap water-sodium carbonate film soil

Residual protein film after washing in

* Represents mean of 3 determinations, µg. nitrogen remaining after washing per mg. film sample (washing as below).

⁺ Represents mean of 6 trials, Aug. nitrogen remaining after washing per mg. film sample (10 min. unagitated wash, 65 to 54° C.).

TABLE 20. - Effect of trisodium phosphate and sodium hypochlorite concentrations on pH of tap water*

Sodium	pH of	trisodium ph at concent	osphate solu ration of	tions
hypochlorite	0.1%	0.2%	0.3%	0.5%
(ml.)	(pH)	(pH)	(pH)	(pH)
0	9.4	10.3	11.0	11.4
2.0	9.7	10.5	11.2	11.5
4.3	10.0	10.5	11.2	11.5
6.6	10.0	10.6	11.3	11.5
12.0	10.4	10.9	11.5	11.6

* Tap water contained 340 p.p.m. total hardness.

TABLE 21. - Evolution of filming procedure to measure protein solubility by the micro-Kjeldahl method

	e	L.	atme	nt 1	CO WÌ	ıich	ri la	LB WE	Le r	ubje	cted	, fi	E E	
bequence of illming operations	A	щ	υ	A	역	fzq	ი	H	н	Ŀ	R	н	W	N
Dipping solutions:														
Tap water (340 p.p.m. hardness)			*н	н	н	×								
Pasteuriz ed skimm ilk	ж	×	×	×	H	×	×	×	H	Ħ				
0.5% NazPO4 in tap water (+2g. CaCl2 + 8 ml. NaOCl)														н
60% clarifier slime in tap water												×	к	H
60% clarifier slime in tap water (+lg. CaCl 2)											н			
0.5% NazPO4 in tap water (+1g. CaCl2)											н	н		
0.5% Na ₅ PO ₄ in tap water (+2g. CaCl ₂)													H	
0.1% Na ₂ CO ₃ in tap water							н	×	×					
1% Ca ₃ (PO4)2 in tap water									н					

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PABLE 21. - Continued

Conners of filmine		Ггея	tmen	tt to	wh	ich.	film	3 We	ເຮືອງ	ıbjec	ted	f1]	B	
operations	A	ъ	ы	Ð	E	F4	ი	н	н	ъ	R	н	M	N
<u>Air drying</u> (over hot plate between dips)	×	×	×	×	×	×	×	×	×	×	×	×	×	×
No. of above sequences for completed film	ц	ŝ	Ś	Ś	10	10	10	20	10	10	10	10	10	10
<u>Irs. drying of completed</u> <u>film @ 80° C</u> .	Ч	ŝ	R	11	4	ĻI	11	11	16	П	16	16	11	13
<u>film weight</u> , mg. (approx.)	2	ω	10	Ø	16	17	11	36	10	18	4	5	Ŝ	9
lg. <u>nitrogen</u> /mg. film sample	•	• •	• •	• •	•	86	101	• •	• •	27	63	95	90	822
<pre>film remaining after 10 min. unagitated wash, 65 to 54° C., pH 11.5 ± 0.1, 0.15% NazPO4 in distilled water</pre>	I	ł	I	I	1	+	+	+	I	+ + +	+	+	+	+ + +

* Indicates treatment.

÷

	<u></u>	Residual protein film	after washing in
Trial		Unchlorinated trisodium phosphate* (ug. N/mg. sample)	Chlorinated trisodium phosphate* (µg. N/mg. sample)
A B C D E F		18.68 14.74 16.17 19.25 23.18 30.31	10.31 10.51 10.74 15.00 17.02 15.71
	x	20.39	13.22
	σ	5.71	3.01

TABLE 22. - Effect of chlorinating (156 p.p.m.) 0.5% trisodium phosphate tap water solutions on protein solubility of soil films

•

* Represents µg. nitrogen remaining after washing per mg. film sample (10 min. unagitated wash, 65 to 54° C., pH 11.5 ± 0.1).

		Residual protein film	n after washing in
Trial		Unchlorinated trisodium phosphate* (µg. N/mg. sample)	Chlorinated trisodium phosphate* (µg. N/mg. sample)
A B C D E		13.17 16.52 15.86 24.46 29.53	15.19 16.11 9.62 16.11 14.96
F G H J		22.19 27.22 24.26 6.59 15.22	8.88 10.33 9.71 6.36 3.08
K L M N O		20.00 7.47 9.01 31.96 10.31	3.19 9.17 8.38 3.59 4.32
	- x	18.45	9.27
	6	7.65	4.65

TABLE	23	-	Effect	of	chlori	nating	<mark>5 (15</mark> 6	5 p.p.n	n.)	0.15%	t	ri-
			sodium	pho	osphate	disti	lled	water	so	lutions	3	on
	.•		proteir	ົສ	olubili	ty of	soil	films				

* Represents ug. nitrogen remaining after washing per mg. film sample (10 min. unagitated wash, 65 to 54° C., pH 11.5 ± 0.1).

TABLE 24 EIIe of s p.p. wate	ct of exposure the oil films washed w m.) 0.15% trisodiu r solutions	ie on protein s with chlorinate m phosphate di	oluoility d (156 stilled
Time	Protein fil	m removed by w	ashing in
(minutes)	Unchlorinat trisodium phosphate (µg. N/mg sample)	ed Ch t p	lorinated risodium hosphate* ug. N/mg. sample)
1 4 6 8	15 17 20 15		15 33 46 67
by washing per m 65 - 1° C., pH 1 TABLE 25 Eval prot 0.15 tion	g. film sample (10 1.5 - 0.1). uation of optimum ein solubility of % trisodium phosph) min. unagitat levels of chlo soil films was ate distilled	ed wash, rination on hed with water solu-
		Residual p after	rotein film Washing
Sodium hypochlorite (ml.)	Avallable chlorine (p.p.m.)	x (µg. N/mg. sample)	σ
0 2 4 6 8	0 78 156 226 312	68.92 55.31 24.42 9.31 7.30	9.8 9.9 14.3 6.4 4.5

* Represents mean of 8 trials, ug. nitrogen remaining after washing per mg. film sample (10 min. unagitated wash, 65 to 54° C., pH 11.5 - 0.1).

	Abso	rbancy of	standard s	amples
Chromium (ug.)	Tri	al l	Trie	1 2
	25 ml. NaOCl (A _s)	50 ml. NaOCl (A _s)	25 ml. NaOCl (A _S)	50 ml. NaOCl (A _s)
0 15 30 50 70 90 120	0.000 0.036 0.075 0.152 0.200 0.241 0.322	0.000 0.050 0.085 0.126 0.187 0.230 0.302	0.000 0.051 0.088 0.132 0.184 0.245 0.289	0.000 0.040 0.090 0.165 0.239

TABLE	26.	-	Standard samples of chromium in chlorinated
			trisodium phosphate solutions* measured by the
			diphenylcarbazide method

* Each standard sample contained 12 g. NazPO4.12H2O, 150 ml. distilled water. Absorbancy was read in an actual or equated volume of 200 ml.

••••••••••••••••••••••••••••••••••••••	Chromium removed*			
Trial	No chlorine (µg. Cr)	l00 p.p.m. chlorine (µg. Cr)	500 p.p.m. chlorine (µg. Cr)	
A B C D	4.0 2.0 4.0	17.0 22.0 26.0 26.0	107.0 136.0 103.0 152.0	
E F G H I	0.0 0.0 2.0 4.0	48.0 47.0 35.0 37.0 31.0	136.0 174.0 168.0 111.0 107.0	
Total	14.0	289.0	1194.0	
ž	1.5	32.1	133.0	
٢	1.7	10.5	27.5	

TABLE	27	-	Chromium losses from type 304 stainless steel exposed to chlorinated 0.3% trisodium phosphate
			distilled water solutions measured by the di- phenylcarbazide method

* Represents µg. Cr. removed from 1120 sq. cm. steel by 48 hour exposure at 47° C.

.

	Absorbancy of standard samples			
Chromium (mg.)	Trial 1		Trial 2	
-	lX-conc.*	2X-conc.*	lX-conc.*	2X-conc.*
	(Ag)	(A _g)	(A _s)	(Ag)
0	0.000	0.000	0.000	0.000
10	0.045	0.040	0.032	0.036
50	0.150	0.155	0.118	0.160
100	0.310	0.315	0.298	0.295

TABLE 28. - Standard samples of chromium in chlorinated proprietary detergent solutions measured by the diphenylcarbazide method

* Represents 2:1 ratio of 2 proprietary detergents (1X-concentration equals 4 ml. of 1st proprietary detergent, and 2 ml. of 2nd proprietary detergent) in 4-1. of tap water evaporated to 150 ml.

TABLE 29. - Amount of chromium removed from 446 feet of 1.5 inch stainless steel pipeline by a chlorinated proprietary alkaline detergent as measured by the diphenylcarbazide method

Trial	Available chlorine (p.p.m.)	Chromium removed (ng.)*
▲	35	0
B	7	0
C	234	1-2
D	56	0
E	56	0
F	56	0
G	49	0
H	42	0
I	49	0

* Represents chromium in a 4-1. sample, taken at an elapsed time of 30 minutes in the washing cycle, $135 \pm 5^{\circ}$ F.



Fig. 1. - Mechanical filming and washing apparatus for gravimetric-micro-Kjeldahl analysis.



Fig. 2. - Temperature controlled mechanical filming and washing apparatus for gravimetric-micro-Kjeldahl analysis.



Fig. 3. - Electron micrograph, 8000 - 10,000 x, of skimmilk film replica.



Fig. 4. - Deposition of skimmilk on 22 x 50 mm. micro cover glasses measured gravimetrically.



Fig. 5. - Skimmilk film formation on 22 x 50 mm. micro cover glasses measured gravimetrically.



Fig. 6. - Absorbancy of films measured by Spectrophotelometer vs. number of times the slide was dipped in skimmilk



Fig. 7. - pensitivity of previmetric vs Spectochotelometer methods of measuring films.



Fig. 8. - Effect of chlorinated buffer solutions at different pH's on protein solubility of soil films.



Fig. 9. - Effect of exposure time on protein solubility of soil films washed with chlorinated (156 p.p.m.) 0.15% trisodium phosphate distilled water solutions.



Fig. 10. - Evaluation of optimum levels of chlorination on protein solubility of soil films.



Fig. 11. - Standard curve showing recovery of chromium in a system containing chlorinated trisodium phosphate when measured by the diphenylcarbazide method. (Average of two trials performed in duplicate)







Fig. 17. - Mean of chromium removed from 1120 sq. or. of type 504 stainless steel by 0.3% chlorinated triscdium phosphate in distilled water at 47° C. for 48 hours.



Fig. 14. - Removal of chromium, as influenced by temperature, from type 304 stainless steel by chlorinated (503 p.p.m.) 0.3% trisodium phosphate distilled water solutions.



Fig. 15. - Standard curve snowing recovery of chromium in a system containing a chlorinated proprietary detergent when measured by the diphenylcarbazide method. (Average of two trials performed in duplicate)

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