"OPTIMIZING FORMULATION PARAMETERS FOR ENHANCED CLEANING EFFICIENCY OF DETERGENTS

Dr Manisha Saxena
Department of Chemistry, Government PG college
Dholpur (Rajasthan)

ABSTRACT

The purpose of this research was to develop a recipe for a dishwashing detergent that was based on alkylpolyglucosides and to maximize its cleaning performance. The formulation of the liquid detergent consisted of the following five components, all of which were of a commercial origin: anionic (linear sodium alkylbenzenesulfonate and sodium laurylethersulfate) and nonionic (C12–C14 alkylpolyglucoside) surfactants; zwitterionic (a fatty acid amide derivative with a betaine structure) surfactants; and sodium chloride for viscosity control. In addition to the plate test, many additional parameters, such as emulsion stability, cloud point, and viscosity, were studied. The creation of a core composite experimental design was carried out with the use of statistical analysis tools. After that, a technique to the design and analysis of experiments known as the Response Surface Methodology was set up in order to study the effects of the five components of the formulation on the researched characteristics in the region encompassing plausible component ranges. This was done in order to determine whether or not the properties could be predicted based on the component ranges. The approach was successful in discovering the domains of concentrations that had the requisite attributes because of its efficiency.

Keywords:*Akyolglucoside, Dishwashing detergent, Formulation, Response surface*

Introduction

In our day-to-day lives, liquid detergents serve many vital purposes, including cleaning personal care items, cleaning domestic surfaces, and cleaning fabric. Liquid detergents have earned a rising market share for a variety of reasons, the most important of which is that they dissolve more quickly than powdered detergents and are simpler to administer. Cleaning performance is a result of the concentrations and kinds of the active substances that are put into the cleaning bath. All other elements, including the surface to be cleaned, the dirt, the temperature, and the hardness of the water, are assumed to be equal. The nonionic surfactants known as alkylpolyglucosides (APGs) have been around for a long time. As a result of the interesting properties they possess and the development of new and more effective technologies, they have been incorporated into detergents, specifically dishwashing liquids. The APGs that are currently manufactured by a number of large detergent companies are derived from renewable raw materials, specifically glucose and fatty alcohols (which come from vegetable oils).

Extensive research has been done to explore their physical and chemical characteristics in pure aqueous or combined with other substances, which often reveal synergistic effects. APGs, in addition to being gentle on the skin, are simply and quickly biodegradable when released into an aerobic aquatic environment, and even exhibit detoxifying properties. They are among the non-ionic species that have the greatest resistance to alkaline. In the current investigation, the objective was to create a hand dishwashing detergent that had four primary components derived from commercial sources. In order to establish a central composite experimental design and explore the impacts of the five components on the chosen qualities of washing performance according to ISO 4198 standardized viscosity, cloud point, and emulsion stability, statistical analysis software was used. This allowed for the development of a central composite experimental design. In addition to having high washing performance, it was desirable for the solution to have a viscosity of at least 300 cSt and a cloud point of less than 5 C. Although just the quantitative elements of the formulation, on a physical and chemical basis, were taken into consideration, psychological and economic considerations may also be crucial when it comes to the market for detergents.

According to the World Health Organization (WHO), health care-associated infections (HCAIs) are the most common adverse event that may occur during the administration of medical treatment anywhere in the world, affecting around 7% of hospitalized patients at any one time. About thirty percent of patients in intensive care units (ICUs) in wealthy nations have at least one healthcare-associated infection (HCAI), whereas the frequency of HCAIs is at least two to three times greater in poor countries. The presence of biofilms, which are composed of blood and other bodily matrices, in medical equipment continues to be a significant barrier to the reduction of the prevalence of When applied to inanimate objects, disinfectants eliminate harmful bacteria or restrict the development of pathogenic microorganisms. One of the markers of the efficacy of a substance is whether or not it has a wide range of antibacterial activity. Additionally, the perfect disinfectant should be compatible with the surfaces that are to be disinfected, simple to produce and use, stable, inexpensive, and easily accessible, and free of any unpleasant odors. They should be effective in the removal of biofilms from medical equipment such as flexible endoscopes, which cannot be disinfected using harsh chemicals or high temperatures, and which may be used to diagnose and treat medical conditions. As a result, detergents and disinfectants that are based on enzymes would be a better choice for use in medical facilities.

These detergents and disinfectants need to be risk-free to use while still being effective, at the very least. At the moment, detergents and disinfectants that may be purchased in poor nations are almost always imported from rich countries. These products, in addition to having problems with stability, convenience of use, and their effectiveness, are also expensive. As a result, it is essential to continue research and development on enzyme-based disinfectants that are efficient and long-lasting, particularly in light of the fact that C. albicans has been identified as a key pathogen in the fungal infections linked with aureus has the capacity to remain in continual

contact with humans and is one of the most significant bacteria that leads to the high frequency of healthcare-associated infections (HCAIs). Disinfecting medical equipment is essential in order to lower the risk of microbial growth and the development of biofilms due to the increased potency of S. aureus biofilms. In light of this, the purpose of this work was to produce an enzymatic detergent/disinfectant that is efficient against C. albicans as well as S. aureus, regardless of whether or not they are present in a biofilm.

OBJECTIVE

1. The Study Optimizing Formulation Parameters For Enhanced Cleaning.

2. The Study The Liquid Detergent Was Formulated With Five Ingredients Of Commercial Origin.

RESEARCH METHODOLOGY

Microorganisms

S. aureus ATCC 25923 and C. albicans ATCC 1023 were the microorganisms that were used in the research that was carried out. They were kept in a freezer that was set at minus 80 degrees Celsius. S. aureus ATCC 25923 strains were cultivated on blood agar plates at 37 degrees Celsius for twenty-four hours while being propagated in trypticase soy agar (TSA). The C. albicans ATCC 1023 strains were cultured on a plate of Sabouraud's Dextrose agar and allowed to grow at room temperature (25 to 26 degrees Celsius) for a period of three days.

Determination of the cleaning efficacy of the formulations

STERIS Animal Health in Derby, United Kingdom developed a product called Brown STF (Soil Test Formula) Load Check Indicator strips, which were used in an experiment to evaluate how well different formulas removed soil. These strips imitate the cleaning effectiveness soil tests for surgical instruments that are defined in ISO/TS 15883-5, and they include two different types of protein in addition to lipids and polysaccharides. In a nutshell, the strips were cleaned at intervals ranging from ten to sixty minutes after being dipped into a dilution of the formulation that consisted of one liter of double-distilled water and six milliliters of the formulation. After being removed from the solution, the strips were cleaned before being photographed. Observing the strips allowed for an evaluation of the amount of time required for the formulation to clear the STF strip. This was seen as a measure of the effectiveness of the cleaning. The interpretation of the data was based on visual observation of the removal of dirt (shown in red in Figure 1), as can be seen in that figure. As indicated in Figure 1, the cleaning effectiveness end-point was regarded to have been reached when the STF strips exhibited a clear appearance.

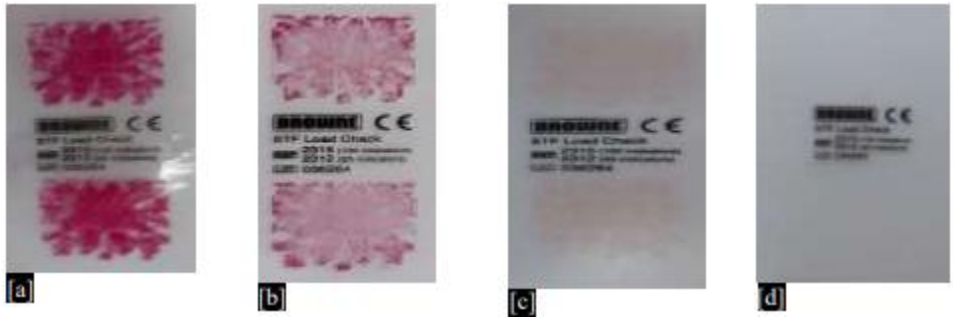

Figure 1. The STF Strips Appeared As [A] With Complete Ineffective Cleaning Efficacy, [B] With Slight Cleaning Efficacy, [C] Moderate Efficacy, And [D]Complete Cleaning Efficacy That Was Taken As The End-Point.

Determination of the disinfection efficacy

A carrier test approach, which was established by G., was used in order to evaluate the antibacterial effectiveness of the improved formulation in relation to S. aureus and C. albicans. After spreading 200 microliters (l) of phosphate buffer saline (PBS) solution over the STF Load Check Indicator strips, the strips were permitted to dry for one and a half hours. The PBS solution included 600 colony forming units per milliliter (CFU/ml) of S. aureus (ATCC 25923) and 9400 CFU/mL of Candida albicans (ATCC 10231). After washing the strips for ten minutes with the improved formulation (six milliliters of the formulation in one liter of double-distilled water), the strips were analyzed. After the strips had been cleaned, a swab was taken of them, and then that swab was suspended in PBS, vortexed, and then tested for sterility on blood agar and Sabouraud's Dextrose agar. Placing a 100 l sample of the contents of the test tube that contained the swab, the mixture was then subjected to an overnight incubation at 37 °C for S. aureus and an incubation at room temperature for C. albicans, respectively. After that, the colony counts of live cells on the plates were determined after they had been disinfected. The experiment was carried out a total of ten times. Following cleaning, the colony counts, as well as the CFU/ml and Log reductions, were calculated. Log reductions were calculated by using the formula Log reduction = log10 A-log10 B, in which A represents the number of viable microorganisms that existed before to disinfection and B represents the number of viable bacteria that existed after disinfection.

A modified version of the procedure described in [7] was used to create the S. aureus biofilm. To summarize, ten aluminum chips with a surface area of 19 cm2 were washed, disinfected with ethanol containing 70%, and then sterilised in an autoclave at 121 degrees Celsius for fifteen minutes. S. aureus was grown in pure culture to a concentration of 2.16 x 109 CFU per milliliter of medium. After placing the chips in the Tryptic Soy Broth (TSB), which contained 60 mL, they were placed in a petri dish that contained 10 mL of the bacterial suspension. The petri dish was

kept in an incubator at 37 degrees Celsius for a total of three days. The chips were then given a PBS wash to clean them. This took place a total of five times. After that, the strips were rinsed with the optimum formulation, which consisted of 6 milliliters of formulation mixed into one liter of double-distilled water, for a range of times, including 10 minutes, 20 minutes, 30 minutes, 50 minutes, 60 minutes, and 24 hours. After the strips had been cleaned, a swab was taken of each one, and then that swab was suspended in PBS and tested for sterility on blood agar. The colony count, colony forming units per milliliter, and log10 reductions were all calculated to determine how effective the disinfection was.

DATA ANALYSIS

Determination of the cleaning efficacy of the formulation

The negative control, which consisted of double-distilled water, was unable to clean the STF strips within the allotted time frame of the experiment (60 minutes). The STF strips were not cleared by formulations comprising just one of the enzymes and no detergent after an experimental duration of sixty minutes. The cleaning time with only the detergent (Sol B) was found to be 35 minutes.

Within a time frame of ten minutes, the STF strips were cleaned when using formulations that included a protease in Sol B (either by itself or in combination with other two enzymes). In under 25 minutes, the protease Everlase alone in Sol A in the detergent (Sol B) proved that cleaning was accomplished. There was no discernible difference in the amount of time required to clean the surface after adding Stainzyme and Lipex to formulations that also included Sol B/EverisDuo and Sol B/Everlase. As can be seen in Figure 2, despite being employed to clean for the longest amount of time allowed by the experiment (60 minutes), aniosyme did not show any evidence of clearing the dirt. It is recommended by the inserts provided by the producer of Aniosyme that the soaking of instruments that are to be cleaned be carried out for a period of twenty-four hours.

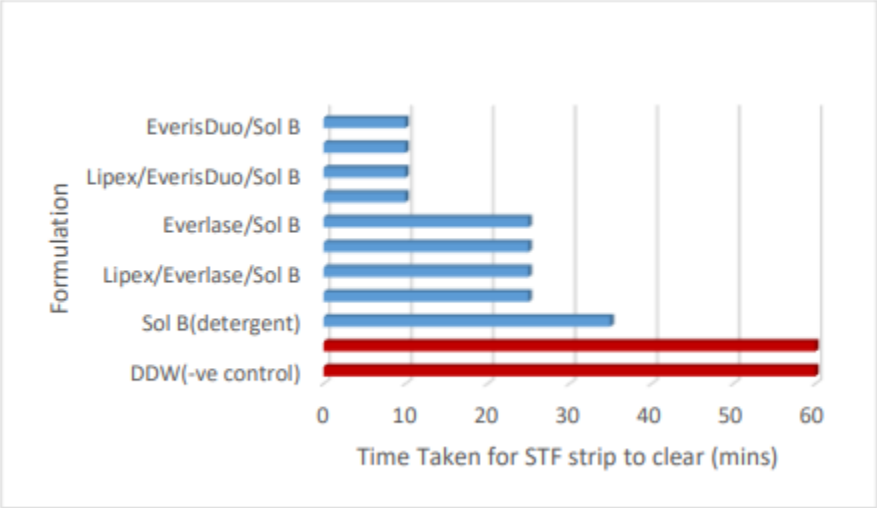

Figure 2. Cleaning Duration Of Various Formulations In Comparison To The Commercial Enzymatic Detergent And Disinfectant (Aniosyme).

The formulations (controls) that did not clear the STF strip within the experimentally determined time period of 10–60 minutes are shown by the red bars in the graph.

The STF strips were cleaned in a period of 20 minutes when Sol B was substituted with the Safikem detergent, which was a superior performance (of 15 minutes) than that of Sol B alone, which removed the STF strips after 35 minutes (Figure 3). On the other hand, the performance of Sol B and Safikem did not change with the inclusion of EverisDuo, which is a formulation that contains enzymes. This meant that the formulations that included tetronic acid may possibly be substituted by ones that contained the chemicals found in Safikem.

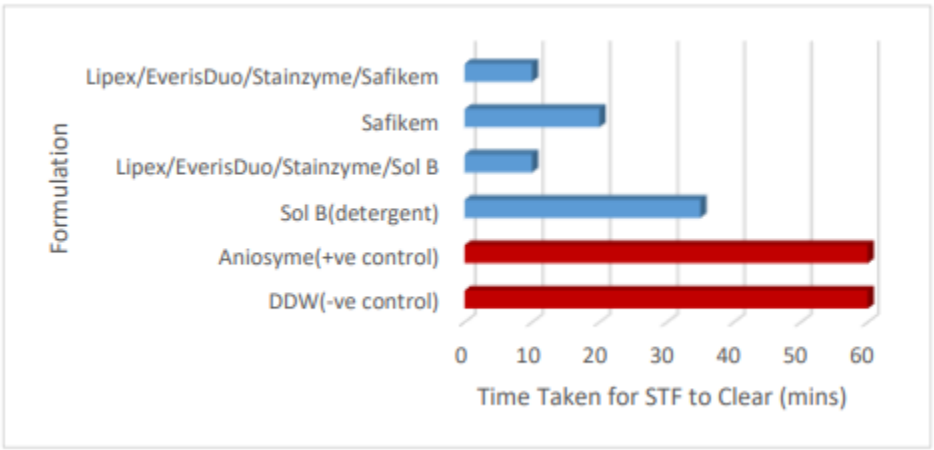

Figure 3. The Cleaning Efficacy Of Various Enzymes (In Detergent) And Safikem. Formulation Containing Sol B (Tetronic Acid 0.3 %), Sodium Bicarbonate, Trisodium Citrate, (Benzalkonium Chloride 0.024 %) And Citric Acid Monohydrous (0.1M) At Ph 8.0 And The Enzymes Everisduo, Stainzyme, Lipex Separately (Sol A). Two STF Strips Were Cleaned Using The KEMRI Detergent (Safikem) As A Positive Control And Double Distilled Water (Negative Control). Red Bars Represent The Formulations (Controls) That Did Not Clear The STF Strip Within The Experimental Set Time Of 10 – 60 Minutes.

Optimization of the key parameters in final formulation of the enzyme cleaner

The cleaning was done using 1 milliliter of detergent or disinfectant prepared in 1 liter of double-distilled water. This was done so that the volume could be used most effectively in 1 liter of double-distilled water. This process continued until the dilution reached 10 ml. The dilutions (6:1 and 7:1) suggested a cleaning duration of 10 minutes, however the dilutions that were lower than 6ml:1liter of water indicated a cleaning time that was greater than 10 minutes. Figure 4 shows that a lower cleaning time is achieved with dilutions greater than 7 milliliters per one liter of water.

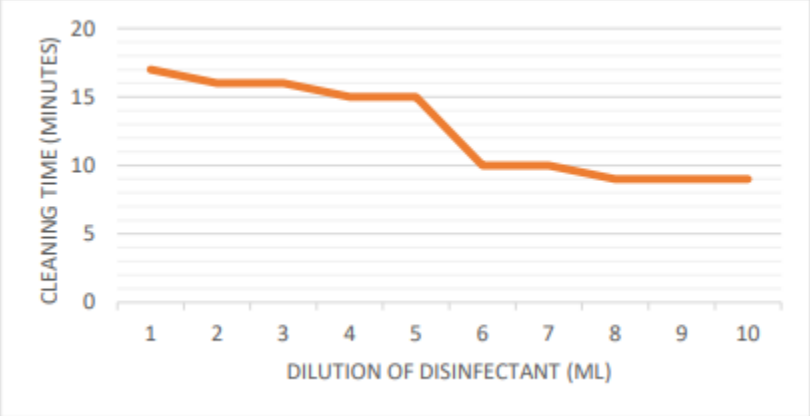

Figure 4 Perfomance Of Cleaning Efficacy Of Various Volume Combinations Of Detergent/Disinfectant (Sol B) In 1 Litre Double Distilled Water To Determine The Most Minimal Effective Volume To Use In Final Detergent/Disinfectant Formulation.

Through a series of experiments aimed at optimization, this research was able to successfully develop an enzymatic detergent/disinfectant that contains Tetronic acid (0.3%), EverisDuo/ Stainzyme/ Lipex, and the disinfectant BAC (1%). This detergent/disinfectant possesses superior performance characteristics in comparison to the currently dominant brand of enzymatic cleanser, Aniosyme. The literature study was relied on heavily throughout the process of determining the preliminary screening of formulation prototypes. According to the results of the investigation, a concentration of BAC of 1% was effective in cleaning the dirt on the STF load

check indicator strip, however greater concentrations were ineffective and hence could not be utilized in the creation of product prototypes. As a disinfectant, a concentration of BAC of up to 3% is suggested; hence, the concentration that was determined over the course of this investigation fell within this concentration. Whoever stated that treatment of medical equipment with benzalkonium chloride decreased C. albicans adherence to plastic surfaces supports the antimicrobial activity of benzalkonium chloride in this research against C. albicans. This finding lends credence to the findings of this investigation.

There was no clearing of the STF strips after sixty minutes when the usual methodology for cleaning the STF strips that was developed in this research was applied using formulations that included at least one enzyme but no detergents. These formulations were employed to clean the STF strips. This might be due to the fact that enzymes on their own are not considered cleansers, but rather are used as catalysts to assist in the digestion of specific dirt found in medical equipment. Because of this, they are added to detergents in the hope that it would increase the effectiveness of the cleaning. In a similar vein, the cleaning performance of detergent by itself was shown to be lower than that of detergent combined with enzyme, which blatantly demonstrates the advantages of mixing detergent and enzyme in order to provide a more effective cleaner. The amount of time (10 minutes) required for cleaning with Lipex/Stainzyme/EverisDuo in Sol B was equivalent to the amount of time required for cleaning with Lipex/Stainzyme/EverisDuo in Safikem. The time required to clear the map of Safikem by himself was twenty minutes. Based on these findings, it may be deduced that the detergent Sol B might be replaced with Safikem.

The findings of this research are in agreement with those of Magazine, which compared the effectiveness of cleaning with non-enzymatic, enzymatic, and alkaline detergents by employing STF load check indication strips in addition to other indicator soils that are well known in the industry. The research showed that employing two different protease types may be beneficial to commercial detergents. The cumulative rank index for using the two enzymatic detergents was 97%, although neither the detergent base nor the alkaline detergent achieved 90%. In this investigation, the inclusion of EverisDuo, which is a protease, resulted in quick cleaning at 10 minutes and was shown to be more effective than the addition of the other enzymes Everlase, which is also a protease, Stainzyme, which is an amylase, and Lipex, which is a lipase. Even though there is not a great deal of published research that explains why some proteases could perform better than others, it is believed that differences in the molecular structures of EverisDuo and Everlase may be the source of the performance gap between the two proteases. Everlase and EverisDuo are both known as proteases. If the quantity of surfactants and volume utilization of the detergent or disinfectant that is used for cleaning is not maximized, this may lead to economic losses owing to excessive manufacturing costs, hence it is vital to optimize both of these factors. According to the findings, a higher concentration of the tetronic acid surfactant is associated with a quicker cleaning of the simulated dirt on the STF strip. This may be explained

by the fact that large concentrations of surfactant lower surface tension, which in turn makes it simpler to moisten the surface and remove dirt.

This research provided evidence of how difficult it is to remove biofilms and ensure that they are sterile. Despite the fact that the cleaning and disinfection effectiveness could be successfully reached after 10 minutes with a log decrease of 7.29 for S. aureus and 5.87 for C. albicans respectively, the same could only barely be achieved after 60 minutes (log reduction of 6.06). After being submerged in water for twenty-four hours, the strips achieved an almost perfect level of effectiveness, with a log decrease of 9.33. This discovery agrees with the findings of another research, which revealed that bacterial spores and biofilms had a lower sensitivity to disinfectants. As a result, in this investigation, exposing the biofilm to the disinfectant for a longer period of time was necessary. In terms of the product's stability, the data that has been gathered up to this point suggests that the product is stable after four weeks with respect to both its pH level and its antibacterial activity. The alkaline pH of the product over time and its capacity to disinfect against S. aureus when employed as a marker for this investigation are in accordance with the findings of those individuals who discovered that alkaline disinfectants had beneficial bactericidal activity. It is thought that the reason why the cleaning of the STF dirt by the prepared detergent/disinfectant became less effective during week 4 is because the STF strip was not exposed to the detergent/disinfectant for a long enough period of time. In order to evaluate the product's ultimate shelf life, we will continue monitoring the stability of the product prototype for the following two years.

CONCLUSION

In the course of this research, an enzyme-based disinfectant that boasts superior cleaning and disinfection capabilities than currently existing items on the Kenyan market was produced. Both S. aureus and C. albicans may be killed by the product, regardless of whether they are in biofilm or not. The newly produced substance is likewise successful in cleaning and disinfecting the bacteria that are present in the biofilm; however, the cleaning process takes much longer, at least sixty minutes. When combined with a tetronic detergent, the research found that a concentration of 1% benzalkonium chloride was effective as a disinfectant when used in conjunction with it.

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