*International Journal on Emerging Technologies* **12**(1): 154-161(2021)

**ISSN No. (Print): 0975-8364**
**ISSN No. (Online): 2249-3255**

# An approach on Enzymatic Synthesised Protein-Based Surfactant for Application in Personal and Home Care Formulations

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*(Received 15 October 2020, Revised 28 December 2020, Accepted 05 February 2021)*
*(Published by Research Trend, Website: www.researchtrend.net)*

**ABSTRACT: Protein-based surfactant (PBS) synthesis based on renewable vegetable oilseed meal protein as a raw material is a solution to waste disposal and broad range functionality surfactant utilization. Enzymatic techniques can be used to endow proteins with surface-active functionality utilizing less solvent and extracted crude rapeseed meal protein when enzymatically. Enzymatic Synthesisof PBS helps to overcome toxicity challenges by using less solvent, environmentally friendly by utilizing renewable resources, and understanding how product application affects personal and home care formulations. Synthesis of PBS reactants Diethanolamine: Protein: Enzyme ratio 20ml: 1.5gm: 30mg optimized for most suitable yield 67.13%. FTIR confirmed the presence of an amino group at 3060-1650 $cm^{-1}$. Surface tension studies of enzymatically PBS can lower water's tension to 33.3 mN/m at 0.5% concentration with Efficient enzyme recovery with five times reuse ability. Basic personal and home care formulations with PBS gave the optimized quality assurance, subjective trials, and antimicrobial studied.**

**Keywords:** Protein-based surfactant, Rapeseed meal protein, lipase enzyme, Enzyme recovery, personal care formulations, homecare formulation

## I. INTRODUCTION

Due to the formation of different amino acids through the peptide linkage, polypeptide polymers give rise to a three-dimensional protein structure. The interlinkages from the hydrogen bonds and side chains of amino acids between peptides lead to proteins' formation [1]. Protein-based surfactants have hydrophobic and hydrophilic portions, modified through chemical or enzymatic means [2]. Linear, branched, or cyclic state of hydrophobic chains generally C8–C25 collectively forms hydrophobic moieties. Whereas atoms O, N, P, S, and the groups -$COO^-$, -$SO_3H$, -$PO_3^{2+}$, -$SO_3^-$, -OH, -SH, -COOH, -$NH_2$ are some of the short polar or ionic portions of hydrophilic moieties interacting with water through dipole-dipole or ion-dipole interactions [3]. The protein-based surfactant can be simply classified into two types of surfactants: 1) Amino Acid Surfactant – Simple amino acids or mixed amino acids derivatives are termed as Amino acid-based surfactants, or they may also be derived from protein hydrolysate. 2) Peptide surfactant – Dipeptides or tripeptides and hydrophobic chains such as fatty acids are condensed to obtain surfactants that are peptide-based. Industrial sectors such as food, agricultural, personal care, home care pharmaceuticals products involve the use of surfactants that affect the surroundings either by manufacturing processes or via domestic use, affecting all aspects of our daily lives. Harsh, severe conditions are necessary for the Synthesis of fabricated surfactant, which has many environmental implications. Hence protein-based surfactants, which are widely applicable in various sectors, can overcome those hurdles. Also, cost and availability considerations make petrochemicals the raw materials of the first choice among manufacturers. Paraffin, benzene, olefin, fatty alcohol, fatty acid, fatty amine, ethanolamine, ethylene oxide, propyl oxide, betaine, and imidazoline, and their polymerized products have been used in petrochemicals derived surfactants [4]. Research can replace these surfactants with protein-based surfactants derived from sustainable resources as they are capable of serving harmless, skin-friendly, profitable, worthwhile, and eco-friendly alternatives. Rapeseed cake is apromising vegetable oilseed cake for Synthesis with high protein excellent protein sources up to 30-40%, while 4.3% ash and 7.9% crude fiber. World wide production of rapeseed mealissecondtosoybeanmeal. Global rape seed meal production was 38.8 million tons in 2018. Vegetable oil meal combustion through various sources may lead to pollution deteriorating the environment; its application as a raw material in protein-based surfactant synthesis would be a potential alternative. Protein from vegetable oil meal can be extracted efficiently by the "alkali extraction and acid precipitation" method [5]. Great significance in economy and society have been given to the application of protein extracted from the oil cake in non-feed and non-food sectors [6] Protein-based surfactants can be synthesized enzymatically wherein Amino acids amides are modified by acylating α-amino group enzymatically using free fatty acids or their methyl esters, studied previously [7,8,9]. Enzymes, commonly lipases, have been used to perform esterification

reactions inboth primary and secondary amine convert to corresponding amine [10]. They are the most important and typical enzymes that function at lipid–water interfaces in micelles, liposomes, emulsions, etc. The unique characteristic of lipases is the "interfacial activation" phenomenon. Since we know enzymes are costly, and considering the cost of Enzyme in Synthesis increases production, Enzyme recovery will greatly contribute to economics. Enzyme recovery is among recent studies in the Synthesis of PBS using immobilized lipases [10]. Improvements of existing personal and home care products by Ampholytic and chelating properties possessing PBS in development of new products with mildness and environmental acceptance [11]. Natural issues, annoyance, and toxicity are among the major factors of encouragement that consumers are now concerned about related to the personal and home care product. Hence, manufacturers seek attention to the selection and production of products containing surfactants with leniency, safety, and tenderness. Enhancing flexibility through absorption of fat like substances, moisture retention capacity improvement via improving skin penetrable, freshening, prevention from skin darkening are the functions to be attained by products containing PBS [12,13].

The present research work aims to replace the surfactant base of formulations from synthetic surfactants with easily bio-degradable enzymatically synthesized protein-based surfactants and increase their potential. Personal and home care market products contain one or the other synthetic surfactant, most common example sodium lauryl sulfate to impart particular property is not environment friendly. Evaluation of personal care products with synthesized protein-based surfactants efficiency was performed.

## II. MATERIALS AND METHODS

*A. Materials*

Novozyme435 taken from the gift sample, Diethanolamine, ethanolamine, triethanolamine, Acetonitrile, Hydrochloric acid, Sodium hydroxide, Isopropyl Alcohol, all chemicals for the formulation of home care and personal care, Nutrient Agar and Bromophenol blue indicator were analytical grade purchased from Hi-Media Laboratories Pvt Ltd. Rapeseed meal was procured from a local vendor from Mumbai, Maharashtra.

*B. Methods*

**Protein Extraction and Estimation.** Proteins from vegetable oil seed cake were extracted using the "alkali extraction and acid precipitation" method and estimation by microKjeldahl method [5].

**Reactants Screening by Amine value.** Screening of reactants was focused on selecting the appropriate quantity for the highest synthesis yield of surfactant. Different amines were used in the screening for the Synthesis of surfactant at different concentrations. A minimal quantity of solvents was used to solubilize the reaction mixture. Acetonitrile and N-hexane were checked for their efficiency. The optimized reaction mixture was kept on the orbital shaker at 200 rpm at 37 for 72 hours [13]. Amine value is the number of milligrams of potassium hydroxide equivalent to the amine basicity in 1g of sample. Amine value determination helps in knowing the number of amine compounds unreacted in the reaction mixture. Take 2.5 g of PBS product mixture in a conical flask. Add 50 ml isopropyl alcohol to it and titrate against the solution of 0.2 N HCl solution in IPA with bromophenol blue as an indicator. Color changes from blue to yellow. This method was repeated until optimized.

$$\text{Amine value} = (BR*56.1*N)/W \quad \ldots(1)$$

Where; BR- Burette reading; N-normality of titrating; w-weight of sample

**Enzymatic Synthesis of Protein-Based Surfactant.** 20ml of Di-ethanolamine and 1.5gm of protein were mixed in a conical flask. To which 30mg of Novozyme 435 was added, and the mixture was placed in a Thermoconstanter orbital shaker at 200 rpm and 37℃ for 72 hours. On the completion of the reaction, the Enzyme used was recovered using the filtration process.The reaction mixture was dissolved in the mixture of chloroform and methanol in a ratio of 50:50 [v/v]. The solvent was then evaporated in a rotary evaporator, and protein-based surfactant that is the product isobtained.

For comparative study, surfactant prepared without Enzyme using a high concentration of diethanolamine. 150gm of Diet hanolamine was heated to 180℃ in 4-necked flask equipped with a mechanical stirrer. To collect reaction water, Dean-stark apparatus was connected. Protein isolate (100gm) was slowly added to diethanolamine in small amounts over a period of one hour. Care was taken to avoid frothing, and the temperature was maintained so as not to drop below 160℃. After the complete addition of protein, refluxing was continued till the aqueous solution of the product showed no precipitate at the isoelectric point of the protein. The reaction mixture was dissolved in 1:1 chloroform: methanol system. After the separation of two layers, the chloroform layer was transferred to a rotary evaporator. The solvent was eliminated by evaporation in a rotary evaporator. The resulting material was amide.

**Enzyme Recovery.** A study on enzyme recovery was also performed to check the reusability of enzymes. After the Synthesis of surfactant in optimized conditions, the Enzyme used was recovered. After the first batch of Synthesis, the Enzyme used was filtered off to separate it from the surfactant. After filtration, it was washed with water and then with acetone and was reused in the next batch.

**Formulations of Personal Care Products.**

**Hair Care Product:** Divide the amount of water into two parts. Part1:-sample + salicylic acid + water; first, add the salicylic acid to water at 25℃. Then add the sample to it. Part 2: - now add NaCl + water. Add part 2 to part 1 at 25℃ till we get fibrous consistency.

**Table 1: Formulation of Shampoo.**

| Sr. No. | Ingredients | Quantity/25 ml |
|---|---|---|
| 1 | Surfactant Sample | 7.5g |
| 2 | Sodium Chloride | 0.75g |
| 3 | Salicylic Acid | 0.125g |
| 4 | Perfume and Color | QS |
| 5 | Water | QS |

**Body Care Product:** Introduce the ingredients in the order listed below in table 2, taking care to homogenize properly after each introduction.

**Table 2: Formulation of Body Shower Gel.**

| Sr. No. | Ingredients | Quantity/25 ml |
|---|---|---|
| 1. | Water | Qs to 100 |
| 2. | Surfactant Sample | 19.2 |
| 3. | Disodium Lauryl Sulfosuccinate | 4 |
| 4. | Cocamidopropyl Hydroxysultaine | 9.6 |
| 5. | Fragrance | 0.2 |
| 6. | Citric Acid | Qs |
| 7. | Preservative | Qs |

**Formulation of Home Care Product**

**Detergent:** All ingredients mentioned in Table 3 are in solid form. Add all ingredients into the mixing tank in the given order and stir the ingredients at 25℃. Continue stirring until all ingredients get homogenized.

**Table 3: Formulation of Detergent.**

| Sr. No. | Ingredients | Quantity (% Weight) |
|---|---|---|
| 1. | Sodium Hexameta Phosphate | **80** |
| 2. | Sodium Metasilicate | **5** |
| 3. | Sodium Tripolyphophate (TPP) | 5 |
| 4. | Sodium Carbonate (Granular) | 10 |

**Floor Cleaner:** Add ingredients given in table 4 to water and mix until homogenous at 25℃.

**Table 4: Formulation of Floor Cleaner.**

| Sr. No. | Ingredients | Quantity (% Weight) |
|---|---|---|
| 1. | Tetrapotassium Pyrophosphate | 20 |
| 2. | Nitinol 5024 | 8 |
| 3. | Stephanie SXS | 2 |
| 4. | DI Water | 70 |

**Oil Displacement.** Oil displacement of synthesized protein-based surfactant was performed. In this test, 50ml of distilled water was taken into the Petri-plate, to which the oil was layered uniformly. To theoil layer, ten microlitres of surfactant sample were gently pipetted out. A clear zone appears, which indicates surfactant activity.

**Foam Stability.** Take 50ml of the solution in a 150ml glass stoppered cylinder. Give 30 vertical strokes. Remove the stopper and foam height measured at different timeintervals.

**Surface Tension.** The surface tension was measured by the Wilhelmy plate method using Kruss Tensiometer K11. Standards were set as follows: standard liquid is water, standard gas is air, 30 readings were taken in 60 seconds at 25℃ plate immersion depth into the samples is 2mm. The study of this was performed for synthesized surfactants, and the results are illustrated in the table.

**Fourier Transform Infrared Spectroscopy (FTIR).** FTIR of the synthesized surfactant was determined using FTIR model Miracle10, Shimadzu. Using a clean capillary, the sample was placed on the spot marked by the infrared light below the tip. A sufficient sample was taken to cover the area lit up by the infrared light. FTIR measurements were performed in transmission mode. IR spectrum was obtained between the range of 450-4500 $cm^{-1}$. The spectrum was studied to interpret the chemical nature of PBS.

**Tergometer Reflectance.** Take a cloth of size 10cm × 10cm. Stain the cloth with the desired stain. If the stain is liquid, dip the cloth for 15-20 mins, after staining dry the cloth for 24 hours, and check the same reflectance. Wash the cloth with the desired concentration of surfactant in tergometer for 15 mins at room temperature and 100rpm. After washing the cloth with the surfactant, rinse it with freshwater for 15 mins. Keep the cloth for drying. Check the reflectance of washed and dried cloth on the reflectancemeter.

Results are expressed in terms of % stain removal using the formula:

$$\%SR = [ (Rw – Ru) / (Rn – Ru) ] \times 100 \quad \ldots(2)$$

Where, R = Reflectance,

n = normal unstained unwashed fabric, u = stained unwashed fabric,

w = stained washed fabric.

**pH determination.** The pH of the products is an important parameter to be maintained. Hence the pH of the product was determined using a pH meter at 25℃. pH can also be used to determine pH.

**Viscosity Evaluation.** The products' viscosity was determined by using Brookfield Viscometer (Model DV-I) set at different spindle speeds from 0.3 to 10rpm. The viscosity of the product was measured by using spindle T95. The temperature and sample container's size were kept constants during the study.

**Percent of Solid Content.** A clean and dry evaporating dish was weighed, and 5 grams of the sample was added to it. The dish, along with the sample, was weighed, and only the weight of the sample was calculated from the total weight, and then the dish was placed on the hot plate for the liquid portion to evaporate. After drying, the weight of dry shampoo that is solids was calculated.

**Wetting Action.** Canvas disk sinking test: canvas disk is floated on the surface of a solution, and the time required for it to sink measured accurately.

**Cleaning Action.** The cleaning activity of the prepared sample was checked. Non-absorbent cotton was wiped with 1 gm of grease and was kept in the conical flask containing the sample solution. Flask was kept on constant stirring for 1 hour. The cotton is removed from the solution, dried, and weighed after 1 hour of constant stirring. The amount of grease removed is calculated using the following formula:

$$DP = 100(1-T/C) \quad \ldots(3)$$

Where, **DP** - Percentage of detergency power, **T** - Weight of grease in a test sample, **C** – Weight of grease in the control sample.

**Moisture Content.** Take 5 grams of the sample placed in a porcelain dish. Dry the sample in the oven and calculate the moisture in the dried sample.

$$\text{The percent by mass} = 100MI/M \quad \ldots(4)$$

**MI**-Loss of mass(g) on drying, **M**- Mass (g) of the material taken for the test.

**Subjective Assessment.** Subjective assessment of prepared personal care products containing enzymatically synthesized protein-based surfactant plays an important role in evaluating different products'

parameters, which will be based on the consumer's personal opinion and interpretation. There is the involvement of the human volunteers, in which they are asked to answer certain questions to know the results of the products after their application. They were asked to answer parameter such as 1) Ease of distribution, 2) Ease of rinsing, 3) Ease of combing, 4) Irritancy

**Antimicrobial Activity.** Sterile nutrient agar plates were prepared. The plate was then divided into four parts and was swabbed with the test organism aseptically. Wells are punched with a sterile cork borer (10mm), and then samples were introduced into the wells, and plates were incubated at 37℃ for 24 hours after incubation, plates were observed for the zone of inhibition and were measured and noted.

## III. RESULT AND DISCUSSION

Petroleum-based surfactants are the most commonly available surfactant whereas surfactant derived from alternative sources such as vegetable de-oil cake is gaining importance considering biodegradability. Vegetable de-oil cake is the residue obtained after the oil that has been extracted from it. They are the most valuable food for a farm animal. Hence, they are rich in protein; they have great importance in the Synthesis of protein-based surfactant. Protein was extracted using the "alkaline extraction acid precipitation "method, and also its protein content was estimated using the MicroKjeldahl method Table 5. Amino acids have two functional groups, the carboxylic and amino groups,whichcan be converted to a surfactant with are active molecule bearing a hydrophobic chain. Amino acids with reactive side chains offer opportunities for the molecular design of Amino Acid Surfactant. Protein is the base for the reaction that contains a mixture of amino acids that are reacted with Diethanolamine which enhances the reaction in biocatalysts. Chemical modification of proteins is not desirable because of the harsh reaction conditions, the nonspecificity, and the difficulty of removing reagents from the final product. Enzymatic modification of protein using Diethanolamine is preferable to chemical modification of protein even though it involves Diethanolamine, which is toxic. Still, the enzymatic Synthesis quantity is less than that of in chemical-based Synthesis, making the enzymatic Synthesis safer, eco-friendly. The enzymatic reaction has several advantages, such as the mild reaction conditions, high specificity, and fast reaction rate.

Moreover, the use of Enzyme is acceptable by consumers from a safety point of view. The addition of amine, such as Diethanolamine toprotein, leads to substituted amide at the cleavage and free amine group site at another end. Moreover, the $H_2O$ molecule is released as the by-product of the reaction.

**Table 5: Yield and Preliminary Analysis of Rapeseed Cake.**

| Parameters | Rape Seed |
|---|---|
| Moisture Content | 10% |
| Protein Content of Rapeseed Meal | 42% |
| Protein Content of Isolated Protein | 85.78% |
| The yield of Extracted Rapeseed Protein | 58% |

The amide formed can be detected and confirmed through FTIR analysis. A particular type of bond vibrates or stretches to absorb energy only when exposed to characteristic frequency radiation. It was noticed that Diethanolamine yields better results than monoethanolamine and triethanolamine. There is only one OH group in monoethanolamine to react with the protein, while in Diethanolamine, there are two OH groups. Hence, less conversion was observed with monoethanolamine than Diethanolamine. While according to literature and previous studies, in the case of triethanolamine, there is a steric hindrance at the carbon linked to amine function, which interferes with the enzyme activity. Hence the Synthesis proceeded with Diethanolamine. Solvents are used to solubilize the reactants as well as the products. Acetonitrile and N-hexane were checked for their efficiency. The reaction was also carried out without solvent for comparison, which showed no difference in the yield resulting from acetonitrile and N-hexane and to that of the batch without solvent. Nevertheless, there are some issues related to the use of the solvents in the reaction, such as it increases the cost of the process, and most importantly, it requires separation of the solvent from the product, which makes the synthesis process lengthier, tedious, and also time-consuming and also acetonitrile like solvent is toxic to some extent.

Hence to overcome those hurdles, the reaction was proceeded without using a solvent. According to the results given in Table 6, it was noticed that the reaction rate was increasing as the concentration was increasing till 30 mg of concentration; once it exceeded 30mg, there was no significant change in there actionrate. Hence the enzyme concentration of 30 mg was used for the Synthesis of the surfactant. The yield was found tobe increasing with the increase in the protein quantity. Hence the highest yield was observed with 2 g of the protein followed by 1.5 g and 1 g of protein. However, the use of a large amount of protein makes the further process of separation moretedious. Therefore,1.5g of protein was used for further studies.

The reaction of crude protein, Diethanolamine, and Enzyme by varying reactant concentration was studied, as shown in table 6. When 30mg of Enzyme was used for batches 1, 2, and 3, the amine value was least in batch two than other batches; this is due to the higher conversion of amine in that batch better yield of the product. The amine value decreased in batch two while it hiked in batch 3. It could be due to the unavailability of active sites for reaction due to a solution becoming thick due to increased protein quantity, and note that there is nouse of a solvent in there action. So, the concentration of crude protein was fixed to 1.5 g for further batches. Certain factors associated with diffusion/adsorption of proteins on the interface affect this is a complicated physicochemical process affected, inefficiency to adsorb, slow dispersion into the interface are several reasons responsible for most of the weaks surface activity native proteins. Thus, a very smaller number of proteins possess surface activity amongst thousands of proteins obtained from the raw materials and agricultural by-products. Low toxicity and biodegradability are the two most considered aspects concerning the surfactants that must be fulfilled by the surfactant obtained from natural oils and proteins. Changing behavior of protein structure and amino acids arrangement are the two factors on which proteins' structure and functions rely.

Proteins are macromolecules that are organized in a sophisticated three-dimensional structure comprising of 20 different amino acid residues. Among the various other Synthesis, Enzymatic Synthesis is preferable due to the increased thermal motion, which balances and maintains native protein structure through rupturing the intermolecular and intramolecular bonds as protein solubility decreases when proteins (especially globular proteins) are denatured.

**Table 6. Results of Reactant Screening.**

| Parameters | Reactant | Ratios | Yield % | Amine Value(mg) |
|---|---|---|---|---|
| Solvent Screening | 5ml Acetonitrile | 20 ml DEA+ 30mg Enzyme + 1.5 g Protein | 68.73 | 232.85 |
| | 5ml n-Hexane | 20 ml DEA+ 30mg Enzyme + 1.5 g Protein | 69.52 | 225.36 |
| | Without Solvent | 20 ml DEA+ 30mg Enzyme + 1.5 g Protein | 67.13 | 242.41 |
| Amine Screening (Without Solvent) | 20 ml MEA | 30mg Enzyme + 1.5 g Protein | 54.62 | 312.56 |
| | 20 ml DEA | 30mg Enzyme + 1.5 g Protein | 67.13 | 242.41 |
| | 20 ml TEA | 30mg Enzyme + 1.5 g Protein | 49.73 | 376.24 |
| Enzyme Screening | 10 g | 5 ml Acetonitrile + 20 ml DEA + 1.5 g Protein | 43.5 | 410.32 |
| (Novozyme 435) | 10 g | 5 ml n-Hexane + 20 ml DEA + 1.5 g Protein | 44.61 | 390.41 |
| | 20 g | 5 ml Acetonitrile + 20 ml DEA + 1.5 g Protein | 49.2 | 340.65 |
| | 20 g | 5 ml n-Hexane + 20 ml DEA + 1.5 g Protein | 51.11 | 330.14 |
| | 30 g | 5 ml Acetonitrile + 20 ml DEA + 1.5 g Protein | 68.73 | 232.85 |
| | 30 g | 5 ml n-Hexane + 20 ml DEA + 1.5 g Protein | 69.52 | 225.36 |

Hydrophobic interactions play dominant roles in the adsorption of surfactants to the air-water and oil-water interfaces. Such a native structure of proteins should be modified to make full use of proteins' surface activity. Unfolding polypeptides and increasing the protein's heterogeneity to diethanolamines, the peptide molecular weight distribution, resulting in improved surface properties, promotes the enzymatic Synthesis**.** The tensiometry measurement of the prepared surfactants was measured using a tensiometer. The efficient reduction in surface tension (SFT) of water confirms surface-active moiety in the prepared surfactants. Surface tension was measured at two different surfactant concentrations, at 0.5% and 1%. The result is shown in Table 7.

The surface tension reducing property of the synthesized surfactants depends upon the chain length of the surfactant. Many authors studied the SFT property of surfactants and reported it in the range of 30-40 mN/m depending upon the protein used for Synthesis. The oil displacement technique relies on the destabilization of liquid droplets by surfactants. Synthesized surfactant sample had surfactant property, the oil drop spread, forming an emulsion with the oil and decreasing the interfacial tension. The stability of drops is dependent on surfactant concentration and correlates with surface and interfacial tension Table 8. The results shown are performed in triplicate and are the mean of three readings ± standard deviation.

The cleaning action of the prepared surfactant was studied using a stained cloth. The cloth was stained with carbon and tea stain and was cleaned intergotometer as mentioned in the procedure. The washing was done using a Terg-O-meter (WadegatiLabequip Private Limited) equipped with a controlled temperature bath system as follows: speed, 100 rpm; washing detergent solution, 1000 mL; washing time, 15 min; rinsing time, 10 min; temperature, 30℃; each surfactant with 0.5% and 1% concentrations was used for washing. The results mentioned in table 8 shows comparative cleaning with SLS, which is commonly used surfactant in laundry application. Although SLS has high cleaning action due to high foam formation, it is unacceptable for machine laundry applications. Due to low foam and moderate cleaning action, protein-based surfactant, and other ingredients of laundry, detergent finds its good application inthe laundry. FTIR graph of the synthesized surfactant shows the amido group and alkyl chain frequency is given, which confirms the formation of amides. Amido group**:** NH stretch was observed in the range of 3070-3350$cm^{-1}$, C= Ostretch in the range of 1590-1750 $cm^{-1}$ and NH bending was observed 1650 $cm^{-1}$. Alkyl chain peak was also seen at 2800 $cm^{-1}$ due to the presence of any reuse of catalyst impacts on the process's economics.

**Table 7: Results of Yield, Concentration, and Surface Tension.**

| Results Surfactants | Yield % | Concentrations (in %) | Surface Tension (mN/m) |
|---|---|---|---|
| Enzyme synthesis PBS | 67.13 | 0.5 | 33.3±0.02 |
| | | 1 | 31.5±0.04 |
| Chemical synthesis PBS | 78.45 | 0.5 | 30.5± 0.03 |
| | | 1 | 28.5±0.03 |

**Table 8: Foam Height, Oil Displacement and Tergometer Reflectance of Enzymatically Prepared Protein-Based Surfactant.**

| Surfactants | Concentrations | Foam Height Stability | Oil Displacement | Tergometer Reflectance (In %) | |
|---|---|---|---|---|---|
| | | (In %) | (In Cm) | Tea Stain | Carbon Stain |
| Enzyme Based PBS | 0.50% | 59.6 | 3.4±0.2 cm | 59.87 | 64.34 |
| | 1% | 64.2 | 4.5±0.2 cm | 62.53 | 69.31 |
| Chemical Based PBS | 0.50% | 75.5 | 4.8 ±0.1 cm | 65.43 | 64.38 |
| | 1% | 82.3 | 5.5±0.2 cm | 71.75 | 75.64 |
| SLS | 0.50% | 92.5 | 4.9±0.1 cm | 73.38 | 76.83 |

**Table 9: Antimicrobial Activity of PBS And Personal Care Product.**

| Sample | Zone of Inhibition (In mm) |
|---|---|
| Protein-Based Surfactant | 32 |
| Shampoo | 21 |
| Body Shower Gel | 29 |

The study of the reuse of catalyst was done to check its activity for reusability. With the reuse of Enzyme in the second batch, a slight decrease in the enzyme efficiency [14] was observed. It was leading to a decrease in the second batch conversion from 67% to 64%. While in the third batch, there was a significant decrease in the conversion to 59%. The further decrease to 45% in the fifth batch, with the loss of the biocatalyst observed during washing and filtering, contributes to the yield's drop.

Chemical and physical properties of formulations based on the application study's primary objective was to determine the efficacy of enzymatically synthesized PBS in-homecare and personal care products. Various properties of the formulated products were studied, sufficient characteristics were attained with respect to the products' foaming. pH is one of the most important factors to be maintained while formulating personal care and home care products. Personal care products with lower pH would lower the level of damage, irritancy to the consumers. Since skin pH is slightly acidic, personal care products with Ph 4.5-7 would be preferred for the best complexion. While most home care products are alkaline, since hydrolysis, chelation, and dispersion of dirt typically occur most effectively at alkaline pH levels. Products contain some solid contents that may interfere with the products' performance if the present will be too hard to heavy to work or hefty to wash out. Then, the water requires wetting agents, i.e., surfactants that modify the system's interfacial tension and enable the aqueous solution to wet the solid surface. Samples showing wetting actionin lesser time are considered to be products with good wetting power.

Wetting action of personal care and home care products was observed in the range of 2-4 minutes, which shows good wetting power. The cleaning action is the primaryaim of home care products and some personal care industry products.

Here cleaning action of formulated products was evaluated for grease, which revealed its potential to clean the grease. The samples' detergency results showed that all the formulations have good cleaning action. The surface tension of the formulations was measured by the Wilhelmy plate method using Kruss Tensiometer K11. Formulated products with synthesized surfactant show a decrease in water's surface tension by the products indicates good detergency power.

Subjective assessment of personal care and home care products play a key role in research and development for personal care and home care products to determine efficacy and safety. Consumers' safety and satisfaction are important for bringing new or improved products to the market. Products formulated involving the synthesized PBS and chemical-based PBS were subjected to different subjective assessments such as ease of distribution, ease of rinsing, ease of combing, irritancy. There must be a uniform distribution of the products throughout the surface where the products to be applied. All the products formulated showed well distribution uring the use of the product.

After applying the shampoo or any other product, it must easily get a wash. After the use of shampoo, combing of hair was tested, which resulted in good combing.

At the same time, verification of irritancy was performed for all the products.

The adverse reaction may occur due to one or the other constituents of the formulation, or it may arise due to the skin's reaction. The reaction may be more severe redness, edema or dryness, etc. There was no irritation observed with any of the formulated products. Overall, all the products showed goods results concerning all the aspects of the subjective assessment. It was determined that the products formulated, including PBS, are shown in Table 10 and subjective analysis in Table 11, possess mildness, gentleness experienced during and after the use of the product. Antimicrobial Activity is one important property of protein-based surfactant is it's antimicrobial. Due to this property, the application of this surfactant in cosmeticing redients proves advantageous as anyother antimicrobial agent in formulation is not needed. The antimicrobial activities were determined 'in vitro' based on the minimum inhibitory concentration (MIC) values, defined as the lowest concentration of antimicrobial agent that inhibits the development of visible growth after 24 h of incubation at 37℃. The prepared surfactant was tested with *E. coli* (*Escherichia coli*) and was found to inhibit the growth of the organism. Results of the antimicrobial activity of PBS and personal care products are presented in Table 9.

**Table 10: Evaluating parameters of prepared formulations.**

| Test Product | Sample | pH Determination | Viscosity (cP) | % Of Solid Content | Wetting Action (Sec) | Surface Tension Measurement (Dynes/Cm) | Cleaning Action (%) |
|---|---|---|---|---|---|---|---|
| Shampoo | C1 | 5.7 | 7490 | 24.8 | 155 | 33.6 | 33.6 |
| | E1 | 5.4 | 7840 | 26.1 | 190 | 32.5 | 30.1 |
| Body Shower Gel | C1 | 5.2 | 8000 | 22.5 | 120 | 32.1 | 52.5 |
| | E1 | 5 | 7982 | 23.9 | 205 | 30.8 | 49.1 |
| Detergent | C1 | 11.8 | _ | _ | 120 | 38.6 | 43 |
| | E1 | 11.4 | _ | _ | 108 | 39.03 | 50 |
| Floor | C1 | 7.8 | 311 | 19.85 | 140 | 41.5 | 40 |
| Cleaner | E1 | 8.2 | 304 | 21 | 135 | 40.5 | 56.8 |

**Table 11: Subjective Assessment of Personal Care and Home Care Products.**

| Product | Sample | Ease of Distribution | Easeof Rinsing | Ease of Combing | Irritancy |
|---|---|---|---|---|---|
| **Shampoo** | C1 | Best | Best | Good | No |
| | E1 | Good | Best | Good | No |
| **Body Shower Gel** | C1 | Good | Good | _ | No |
| | E1 | Best | Best | _ | No |
| **Detergent** | C1 | Best | Best | _ | No |
| | E1 | Best | Best | _ | No |
| **Floor cleaner** | C1 | Good | Good | _ | No |
| | E1 | Best | Best | _ | No |

## IV. CONCLUSION

Protein-based surfactants prepared using diethanolamines constitute bio-based surfactants with surface-active properties, wide biological activity, low potential toxicity, and low environmental impact. Moreover, they can be prepared efficiently by clean biotechnologies such as enzymatic catalysis. All these features make them an outstanding alternative to conventional specialty surfactants. The awareness and need for cosmetics with natural ingredients are on the rise, as it is strongly believed that these products are safe and free from side effects.

## V. FUTURE SCOPE

This research is a step taken in utilizing the natural abundant vegetable oilseed cake resource to rely less on petroleum products. Also, synthesizing surfactant and developing commercial eco-friendly products that will be highly acceptable was the principal concern. Such acceptable results envisioned applications for protein base surfactant for specific end-use. A broad range of functionality is desired, such as safe, mild, surface activity, biodegradability. Enzymatic Synthesis gives several benefits, such as faster reaction rates and high specificity. The research concentrates on accelerating this rare surfactant class that's ecosystem friendly but lacks research due to the complex nature of the protein. Proteins are amphipathic, and if they are modified or altered using Enzyme can have surface-active properties are called protein-based surfactant.


## ACKNOWLEDGMENT

The authors wish to acknowledge the Institute of Chemical Technology, Mumbai, for availing facilities and the Rajeev Gandhi national fellowship for S.C. for funding the project.


## CONFLICT OF INTEREST

The authors do not have any conflict of interest

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**How to cite this article:** Wanjari, N., Bhalekar, G., Siddiqui, Mohd. Asif and Waghmare, J. (2021). An approach on Enzymatic Synthesised Protein-Based Surfactant for Application in Personal and Home Care Formulations. *International Journal of Emerging Technologies*, *12*(1), 154-161.