COMPARATIVE INTERACTION STUDY OF LIPASE AND SURFACTANTS

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelor's of Technology

In

Biotechnology

By

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to

Department of Biotechnology and Bioinformatics

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DECLARATION

I hereby declare that work presented in the thesis, entitled" COMPARATIVE INTERACRION STUDY OF LIPASE AND SURFACTANTS" for the partial fulfillment of requirements for award of degree of Bachelor in Biotechnology & Bioinformatics submitted in department of Biotechnology & Bioinformatics Jaypee University of Information Technology, Waknaghat is an authentic record of my own work carried out over period of 2019-2020 under the supervision of Dr. Poonam Sharma , Department of Biotechnology & Bioinformatics.

The matter embodied in this thesis is my original work and has not been submitted for award of any other degree or diploma in this or any other university/institute.

Palak Sharma

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CERTIFICATE

This is to certify that the work titled "COMPARATIVE INTERACTION STUDY OF LIPASE AND SURFACTANTS" submitted by Palak Sharma (161826) for the partial fulfilment for the award of degree of Bachelor's of Technology in Biotechnology to Jaypee University of Information Technology, Solan (HP) is a bonafide research work under my guidance and supervision. No part of this thesis has been submitted for any other degree diploma in any university.

The assistance and help received during the course of investigation has been duly acknowledged.

Place: Waknaghat

Date: 16/07/2020

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LIST OF SYMBOLS AND ACRONYMS

BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
CMC	Critical Micelle Concentration
$\Delta H°m$	Standard enthalpy change
$\Delta G°m$	Standard Gibb's free energy change
$\Delta S°m$	Standard entropy change
ϒ	Surface tension
SDS	Sodium dodecyl sulfate

ABSTRACT

Proteins are widely used in current detergents; however there are not too many tests that control the amount of chemicals in proteins. Catalyst-based lubricants have been used to redefine the intended cleansing bath dispensers. This is an investigation of the facilities in the immediate vicinity of making a cleaner, more efficient and productive cleaner with the help of chemical lipase and monitoring its interaction with wetting agents i.e. SDS, CTAB, Tween-20. The various experiments are directed at four diverse points i.e. 25^{o} C, 30^{o} C, 35^{o} C, 40^{o} C. The physio chemical properties are evaluated to determine the basic concentration of micelle (CMC), thermodynamic parameter (ΔH^{o} m, ΔS^{o} m, ΔG^{o} m) Sodium dodecyl sulfate (SDS), Tween- 20 and cetyl trimethyl ammonium bromide (CTAB). Pressure readings were also performed with Sodium dodecyl sulphate (SDS), Tween- 20 and CTAB.

1.1 DETERGENTS

Detergents/Cleansers containing impetuses are suggested as "green chemicals" inferable from their biodegradability, low harmfulness, non-ruinous tendency, condition suitability. [1,2] Cleansers when in doubt fuse six substances: surfactants, producers, proteins, passing on experts, fillers and elective minor included substances [3,4]. Usage of synthetic compounds in chemicals licenses lesser temperatures that is used, less time of tumult are vital.[5] Surfactants trade with proteins and their arrangements, change discretionary arrangements of polypeptide chains of proteins in view of different electric charges and assorted hydrophobicity.[6] Surfactants accept an imperative occupation in game plan of chemical definition. In such way we study the physico-blend effects to get the thermodynamic parameters of wetting agents i.e SDS, CTAB, Tween-20 and also determine fundamental micelle center(CMC) in proximity of protein lipase at different heats 25^{o} C, 30^{o} C , 35^{o} C , 40^{o} C. The assessment also joins dynamic examination, tally of dynamic parameters, surface strain considers have moreover been directed to lipase to check effect of lipase on surface weight of surfactants.[7]

1.1.1 MODE OF ACTION OF DETERGENTS

The purifying activity of cleanser results from its capacity to bring down the surface pressure of water, to emulsify oil or oil and to hold them in a suspension in water.

- The capacity is because of the structure of cleanser. Cleanser/cleanser anion comprises of a long hydrocarbon chain with a carboxyl gathering at opposite end.
- The hydrophobic end is solvent in oils.
- The hydrophilic part is solvent in water.
- In water, cleanser disintegrates to frame cleanser anions and sodium cations.
- Similarly the anionic piece of a cleanser likewise comprises of a hydrophobic and hydrophobic end.
- As cleansers brings down the surface pressure of water, the mechanical tumult assists with pulling oil or oil away from the fabric and oil is broken in littler parts , along these lines material is cleaned .

1.1.2 LIPASE

Lipase play out a basic job in assimilation, transport and preparing of dietary lipids. Most lipids act at a particular situation on glycerol spine of lipid substrate. Lipases are engaged with differing natural frameworks going from routine digestion of dietary triglycerides to cell flagging and irritation. Hence, a few lipases are kept to explicit compartments to cells while others work in the extracellular spaces.

1.1.3 SURFACTANTS

Surfactants are bothers that lower surface strain between two liquids, among air and a liquid. Wetting agents exists in regularly normal disturbs which are amphiphillic, that infers they contain both hydrophillc(heads) and hydrophobic (tails). As such it contains both water – dissolvable and water-insoluble parts. In the form fluid state, surfactants structure sums, for instance, micelles, where the hydrophobic heads are in contact with the including liquid. Various sorts of aggregates can similarly be encircled, for instance, round or tube formed micelles. The condition of complete depends upon substance construction of surfactants, essentially the adjustment in size between the hydrophilic head and hydrophobic tail. The different sorts of surfactants are:

• Anionic Surfactants :

The hydrophilic part of the ionic surfactant has a negative charge. The negative charge allows the surfer molecules to propagate into the micelles and reach the ground. Anionic surfactants are commonly used in rinsing because they can invade a wide range of soil. Anionic surfactants combine to form large amounts of foam. If ionic surfactants are unreliable to entice and interconnect the earth, then it is unacceptable to combine them with greasy earth.

Examples: Sulfates, sulfonates and gluconates

• Cationic Surfactants :

Cationic surfactants have a positive charge on the hydrophilic part. Positive charge can compare these valuable items with purging agents. Cationic surfactants can also be filled as antimicrobial specialists, so they are routinely used as sanitizers. Cationic surfactants cannot be used with ionic surfactants. Considered jointly and unfavorably charged, they exit the arrangement and are not viable at this time. But in case of cationic and non-ionic they can be used together.

Examples: Alkyl ammonium chlorides

• Non – Ionic Surfactants :

These surfactants are always unbiased. Non – ionic surfactants are truly adept at combining emollients and are superior to anionic surfactants at expelling natural earths. The surfactants are normally utilized together to make a double activity. Certain nonionic surfactants can be less frothing. This settles on them a decent decision for less-frothing cleansers. Nonionic surfactants have a one of a kind assets termed a cloud point. The cloud point is heat at which the nonionic surfactant starts to isolate from the cleaning arrangement, called stage division. At the point when this happens, the washing arrangement turn out to be overcast. This is viewed as the point for ideal detergency. The point of the cloud point relies on proportion of hydrophobic and hydrophilic bits of nonionic surfactant.

Examples: ethoxylates, cocoamides

• Amphoteric Surfactants:

Amphoteric surfactants have a twofold charge on their water repelling end, both negative as well as positive. Twofold charges balance the other creating a net charge of zero, insinuated as zwitterionic. The pH of any course of action chooses how the amphoteric surfactants react. In low pH game plans, the amphoteric courses of action turn out to be insistently charged and continue correspondingly to cationic wetting agents. In stomach settling agent game plans, they make a negative charge, like the anionic wetting agents. Amphoteric wetting agents are normally useful for near and dear thought things, for instance, shampoos and embellishing specialists.

Examples: betaines, amino oxides.

1.1.4 SURFACTANTS USED

A) TWEEN-20:

Polysorbate 20 is a nonionic surfactant denoted by the ethoxylation of sorbitan before the expansion of the lauryn corrosion. Its sound and non-toxicity allow it to be utilized as cleansing as well as emulsifying agent around various residential and logical applications. Name 20 leaves the process of ethoxylation with repeated polyethylene glycol.

B) SODIUM DODECYL SULFATE (SDS)

SDS is an ionic surfactant used in many washing and sterility products. This quota is an organosulfate and salt. It has a 12-carbon tail close to the sulfate group. Its hydrocarbon tail composite with polar headgroups gives amphiphilic properties and can therefore be used as a laundry detergent. SDS is not cancerous when delivered or applied directly.

C) CTAB

CTAB forms micelles in aqueous solutions. It is also used in antiseptic drugs and is also very effective against antimicrobial activities. CTAB is slightly toxic in bulk and has also been used in the synthesis of nanoparticles.

1.2 PHYSICO- CHEMICAL PROPERTIES

The chemical and physical changes that are there in development in assembly of atoms and molecules and their contact. Some of physic- chemical properties that were studied includes: conductivity, surface tension, boiling point, CMC , micellization ,all of them having a noteworthy role in pre formulation studies.

1.2.1 CONDUCTIVITY:[8]

It is a perfect material conducting electricity. This is intended as the ratio of the current density in the material to the electric field, which causes the current to flow. Siemens per meter is SI Unit of Conductivity (S / m).

1.2.2 SURFACE TENSION:

Surface strain aids with comprehension of living responses occurring in the arrangement as well as on a superficial level and interface. Surface strain is characterized by way of the powers of fascination applied on the exterior atoms of a liquid by the particles below that will in general bring the surface atoms into the mass bringing about the liquid accepting the shape with least exterior region. Least exterior zone is vital by a liquid for remaining in the most minimal vitality state. The SI unit of surface strain is Newton/meter (N/m). Knowing about surface strain empowers definition of detergent.

1.2.3 BOILING POINT:

The boiling point is the temperature where the fume mass of a liquid equals the mass surrounding the liquid and the liquid is turned in vapor. Boiling point of a liquid fluctuates depending on the surrounding natural mass. Liquid inside an incomplete space has a lesser boiling point than when that liquid is at air pressure. A liquid at more mass has a upper boiling point than when that liquid is at barometrical mass.

1.2.4 MICELLIZATION:

They are aggregates of wetting agent particles scattered within the fluid suspension. The total water in the liquid system is designed to divert the waterhead areas, to align the direction, and to separate the endpoints that remove the water in miles. Structural micelles usually add to the amphibians in the atmosphere, indicating that they are dissolved as solvents in water and as liquids in acetone. The pioneering forces are the result of consistent correspondence between the polar heads and the end result of electrostatic cooperation, the effects of water on polar free tails and major unstable forces. Micelles are usually round-framed cells that run from 2 to 20 nm in size.

1.2.5 CRITICAL MICELLE VALUE:[9,10]

Critical micellar values are described as the primary focus on which micelles begins to develop. It can be solved using conductivity, electrophoresis, surface tension, dispersion techniques, voltammetry. It is believed that the CMC of a wetting agent is an essential imperative for determining the various barriers to searching for use in the medical profession, considering contextual manifestations. In this way, micellar calibration can be used as a medium to achieve an ideal utility based on different particle temperature, pH, focus and inflation.

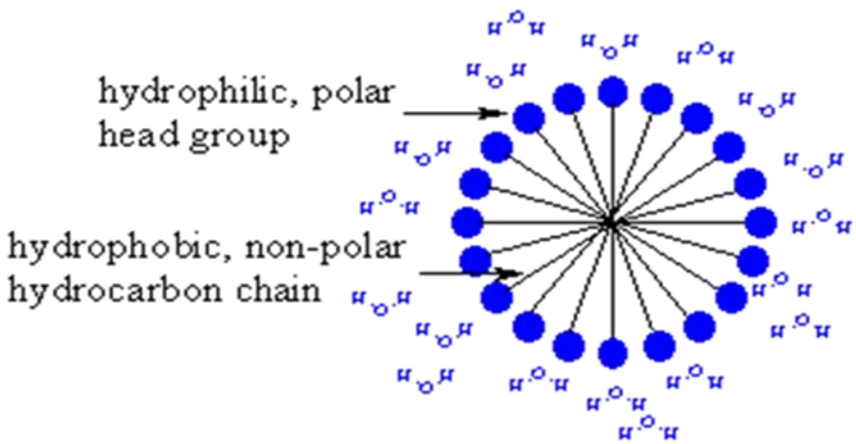


FIGURE 1: Representation of Micelle Structure

THERMODYNAMIC LAWS AND THERMODYNAMIC PARAMETERS

A) THERMODYNAMIC LAWS

- **First Law:**

. When energy passes as heat enters or exits the system, the internal energy of the system changes according to the law of conservation.

- **Second Law:**

With the natural thermodynamic process, the number of active thermodynamic inputs is increasing

- **Third Law:**

The system entropy approaches the average rate as the heat advances towards zero completely. With the exclusion of solids that are non-crystalline, the whole system penetration is close to zero.

- **Law of conservation of energy**:

This law tells that the absolute power of a single system is permanent. Power cannot be created or destroyed but can only transmit from one form to another**.**

B) THERMODYNAMIC PARAMETERS

Thermodynamic parameters remember variation for change in Gibb's free energy (ΔG), change in entropy (ΔS) and change in enthalpy (ΔH). These constraints are valuable in significant response and the way researchers are able to accomplish an ideal pace of response by specific modifications of the focuses.

•Enthalpy:

A thermodynamic value that is exactly the same as the complete warm element of the frame. In terms of numbers, $H = U + PV$ (H denotes enthalpy, U speaks to the dynamism within the frame and P, V speaks to weight and capacity separately).

•Gibb's free energy:

A thermodynamic value that’s considered to be inaccessible to the thermal energy of the transformed frame is a mechanical function. It is as if rather, it is the degree of consistency of the formed particles or atoms in a framework.

•Entropy:

A thermodynamic value such as the enthalpy of a frame is a result of entropy and total temperature, for example $\Delta G = \Delta H - \Delta ST$ (where ΔS represents the change in entropy).

2. AIM

The primary point of the study was to plan a detergent that doesn't influence condition and is a green detergent. The distinctive physico-compound properties were utilized in definition of detergent.

The specific objectives of study were:

1) To calculate thermodynamic parameters of enzyme-surfactant interaction with help of following physical properties:

- Conductivity
- Surface tension

2) To carry out Degradation studies:

- Biological Oxygen Demand
- Chemical Oxygen Demand

* All the studies will be performed at 4 diverse heats (25^{o} C , 30^{o} C, 35^{o} C , 40 ^{o} C)

3.1 REVIEW OF LITERATURE

Broad writing survey was concentrated to acquire information about lipase and surfactants utilized (CTAB, SDS and Tween-20) and an understanding into the labor put in by different researchers or inventors on the equivalent, and details.

- **Comparative interaction study of amylase and surfactants for potential detergent formulation:**

The main objective for this paper is to make a green detergent using amylase and surfactants which is efficient in cleaning also. The enzyme that is used helps in increasing efficiency of detergent and making it more eco – friendly & low toxic detergent. It is referred to as green detergent because of enzyme being used for formulation of detergent. Different studies are done on enzyme & surfactants together to check its efficiency and if combination of surfactants and enzyme go together.

- **Thermo- acoustical analysis of SDS:**

Fluconazole-based micellar framework in fluid-ethanol responses to novel claims of possible drug status. From conduction and thermodynamic investigations, micellar system dependence and inconsistent methods have been observed in the inflation of FLZ at specific temperatures.

- **Micellization, interaction and thermodynamic study of butylated hydroxyanisole and sodium dodcyl suphate in aqueous –ethanol solution at 25, 30 and 35 C:**

The surfactants are found to upgrade dispersion relying upon hydrophobic/hydrophilic length and structure of the surfactant atom. In this paper, the collection properties of SDS in nearness of butylated hydroxyanisole had been estimated at various temperatures 25, 30, 35 ^{o} C in a watery – ethanolic compound mixture. In information given the fluid – ethanolic arrangements as per a component of surfactant SDS focus on extending that shows nearness of micelle formation and collaboration systems. Distinctive thermodynamic parameters were assessed and variety in these shows that with change in temperature there is an appearance of hydrophobic communications.

•Effects of Surfactants on Lipase Structure, Activity, and Inhibition: (Published on fourteenth January 2011) [11]

In this paper it has been seen that lipase inhibitors are principle hostile to weight drugs given , the intricacy of its structure makes it hard to shape new atoms . It is for the most part dependent on physico-science of its lipid-water interfaces. Lipolysis happens in oil-water media and includes complex equilibria among adsorption and desorption forms, surfactants can help in actuating noteworthy changes in parceling of catalyst and halt amid the liquid stage and protein - water stage. Wetting agents are likewise set up at emollient-liquid line where they rival adsorption and furthermore in arrangement structures micellae totals that connects with hydrophobic pieces of lipase. These different associations, joined through the emulsifying properties and scattering of insoluble substances and stopping agents, can whichever advance or lessening movement and hindrance of proteins.

• Interaction of enzymes with surfactants in aqueous solution and in water-in-oil microemulsions

(Published in 1988) [12]

The dynamic investigations were completed on hydrolysis responses did by an assortment of lipases in watery arrangement in nearness of surfactants to discover the job of surfactants in procedures of microemulsions. The fundamental ends were that the impact of surfactants on lipase plays a job and it was reliant on wellspring of lipase. Serious and non-serious restraint was identified with AOT, CTAB and SDS. In everyone of the cases, hindrance was alterable yet it was additionally observed that proteins are impervious to unhurried denaturation by wetting agents over a time of a few periods. It was seen that there was no relationship between's the conduct of chemical in fluid arrangement of surfactants and conduct in liquid-in-oil micro blends balanced out by CTAB and AOT.

- **Hard- Surface Cleaning Using Lipases: Enzyme–Surfactant Interactions and Washing Tests:**

(Published on 16th January 2007) [12]

A business lipase Thermomyces lanuginosus was concentrated so as to survey collaboration per business nonionic and anionic surfactants, just as its cleaning activity applied by chemical on hard surfaces. It was seen that nonionic surfactants forestalled chemical infiltration at the interface, in this way diminishing the lipase movement, there is no inhibitory impact of anionic surfactant was found on protein. There was a upper change accomplished in enzymatic hydrolysis after 20 min within the sight of wetting agent than in lack of wetting agent. The shower substrate-stream was utilized for washing tries different things with lipase at various temperatures with and without surfactants.Two distinctive slick stains it was discovered that lipase without anyone else builds detergency fundamentally, forestalling the ensuing redeposition of evacuated earth.

•The Surfactant-Induced Conformational and Activity Alterations in Rhizopus niveus Lipase:

(Published on 26th November 2014) [13]

The impact of nonionic , zwitterionic, cationic, anionic cleansers on enzymatic action and basic steadiness of lipase was seen. The basic changes were observed by a wide margin – UV CD that shows surfactant prompts helicity in protein which was highest in CTAB , after that SDS, then CHAPS and then Brij-35.The basic variations were checked by tryptophan fluorescence. The enzymatic dynamic measures were likewise run that indicated that action was improved by means of 1.5 and 1.1 crease with nearness of CHAPS and Brij 35 respectively. At hand there is an additional decrease in movement of 20-30% if there should be an occurrence of SDS and CTAB.

•Influence of Surfactants on Lipase Fat Digestion in a Model Gastro-intestinal System:

(Published on 26th January 2006) [14]

In the current day, a typical gastro-intestinal examination is utilized on the way to consider the impact of various nutrients on lipase action. Pendant drop strategy is utilized to locate the interfacial movement of lipase and surfactants. A scientific model is embraced that empowers quantitative assurance of arrangement of liquid-oil interface as a component of mass wetting agent focus in liquid-oil blends. There was a decline in triglyceride hydrolysis all through gastro-intestinal tract. It was inferred that lipase can disorp Sn-2 monopalmitin, which along by prohibition of surface from edge clarifies about step by step diminishing triglyceride hydrolysis that happens during the processing.

•Interaction of Rhizomucor miehei Lipase with an Amphoteric Surfactant at Different pH Values:

(Published on 29th October, 1997) [15]

Utilizing surface strain and ellipsometry the collaborations of surfactants with compound Rhizomucor meihei lipase were considered. From past surface strain examines its seen that didocylmethylammonium bromide shapes a total by means of R.miehei lipase , though surfactants don't frame these compound. In this examination, the impact of the surfactant accuse on complex arrangement of protein is researched utilizing the surfactant N-sarcosinate. It was affirmed that lone when the head bunch was decidedly charged that catalyst bound to surfactant. It was seen underneath the basic micelle focus restricting happens with adversely charged destinations on the protein cooperating with cationic surfactant head gatherings. It was accepted that water repelling area nearby the adversely charged site of the protein empowers a water repelling site fascination between the catalys and cationic surfactant.

•Reverse micelles as reaction media for lipases:

(Published on November, 2000) [16]

Reversed micelles have been related with thought of a microreactor when compounds are shielded from dissolvable impeding impacts. This paper features some of principal parts of lipase microencapsulation and turned around micelles framework. The properties of micellar condition is watched and identified wiith lipases' in term of movement and solidness. The heterogenicity of turned around micelles are likewise watched.

•Inhibition of lipase-catalyzed hydrolysis of emulsified triglyceride oils by low-molecular weight surfactants under simulated gastrointestinal conditions:

(Published on 2nd October, 2011) [17]

The investigation was on impact of low-sub-atomic mass surfactants on the absorbability of fats in protein-balanced out corn emollient-in-water suspensions utilizing an absorption classic, the effect of nonionic , anionic and cationic surfactants on amount and degree of fat processing was additionally studied. It was discovered wholly surfactants repress lipid assimilation at adequately high focuses .The capacity of the low-sub-atomic weight surfactants to restrain lipid processing was ascribed to numerous potential mechanisms.Many of surfactants were discovered helpless to catalyst absorption without lipase.This study has significant ramifications for food and pharmaceutical applications.

•Increased Activity of Chromobacterium viscosum Lipase in Aerosol OT Reverse Micelles in the Presence of Nonionic Surfactants :

(Published on September ,1993) [18]

In this investigation, to progress the movement of Chromobacterium viscosum lipase the adjustment of the switch micelle frameworks through different alkyl glucosides and nonionic wetting agents was tried. Tweens and Tritons supposedly was soluble at emollient-liquidedge of micelles. Alkyl glucosides were likewise soluble at edge. It was seen that expansion of nonionic surfactants diminished the hydrophobicity of converse micelle frameworks and there was additionally an improvement in lipase action along these lines.

•Ester synthesis in lipase-catalyzed reactions:

(Published on 28th April, 1998) [19]

In this investigation, bunch of response frameworks were utilized in ester creation catalyzed by lipases. The chemicals conduct in response frameworks is because of certain auxiliary examples of lipases. Water apparently has an impact on lipase conduct either legitimately by influencing hydration of the compound or in a roundabout way by changing the idea of media. In expansion to water , there are numerous different elements that influence the idea of media. Many of techniques for water evacuation and their adequacy are examined in this examination.

•Lipase – Surfactant Interactions :

(Published on 12th January 2008) [20]

In this examination, the connections between various amphiphiles and Rhizomucor meihei were invistigathed utilizing different strategies. By estimating the portability of emulsion beads and ellipsometry the cooperations at the emollient-water and strong liquid interfaces were inspected. It was discovered that the cationic wetting agents structures compound with protein lipase for a wide scope of pH. It is likewise observed that connection amongst cationic wetting and lipase is because of a blend of the electrostatic fascination and water repelling association and that there is no consolidated communications happens with cationic wetting agents. The collaboration between cationic surfactants and protein lipase prompts decrease of response rate in lipase catalyzed hydrolysis.

4.1 MATERIALS

4.1.1 Enzyme Purchased: Lipase was attained from **HIMEDIA** Labs Pvt Ltd, India.

Table 1: Common Lab Materials and Glasswares

S.No	**Glassware's and other common materials**
1	Beakers – 25, 50 and 100 mL
2	Measuring Cylinders – 10, 100 mL
3	Pipettes
4	Specific gravity bottle
5	Centrifuge Tubes
6	Ice bucket
7	Distilled water
8	Aluminium foil

Table 2: Equipment and Apparatus Used

S.No	**Equipments and Apparatus**	**Manufacturer**
1	Weighing Balance	Citizen (CY Series)
2	Conductivity Meter	Eutech Instruments
3	Water Bath	Relitech
4	Ostwald's Stalagmometer	HARCO

4.2 METHODOLOGY

4.2.1 Stock Solution Preparation: Three different stock solutions were prepared for CTAB, Tween-20 and SDS respectively.

A sample solution with distilled water and 1% lipase was made.

A small amount of stock solution was added stepwise to sample solution and readings were taken.

- Lipase in 1% concentration was taken and Tween 20 concentration was added in small installments (1mM- 13mM) from stock solution at diverse heats (25 º C, 30 º C, 35 º C, 40 º C).

- Lipase in 1% concentration was taken and CTAB concentration was added in small installments (1mM-17mM) from stock solution at diverse heats (25 º C, 30 º C, 35 º C, 40 º C).

- Lipase in 1% concentration was taken and SDS concentration was added in small installments (1mM-14mM) from stock solution at diverse heats (25 º C, 30 º C, 35 º C, 40 º C).

4.2.2 Conductance:

The conductivity was measured by innersing the probe of the equipment in a beaker containing the sample solution, the probe was dipped to a certain point in solution. The conductivity value shown in conductivity meter was recorded at diverse temperatures (25 º C, 30 º C, 35 º C, 40 º C) containing diverse concentrations of wetting agents i.e CTAB, SDS and Tween-20.

4.2.3 Surface Tension:

(i) Stalagometers with an elastic cylinder and a screw squeeze roster were used to determine the surface pressure of the surfactants. Advances have been made in determining surface pressure:

(ii) fasten the stalagmometer and slow the bolt squeeze roster, allowing 15-20 drops every moment.

(iii) A delay in time taken for drop to fall in beaker was noted and the drops that were counted were used in determination of surface tension.

(iv) Repeat the procedure for different arrangements of lipases with different surfactants at 25antsC, 30º C, 35º C, 40º C.

(v) The thickness of the compound and the alignment of the surfactant shall be determined with the help of a clear gravity bottle and an adjustable gauge balance. The following formula is used to find the surface tension:

$$ɣs = (ds.\ nw/ds.ns) \times ɣwdyne/cm$$

where,

nw : number of drops of water

dw : density of water at temperatures (25^{o} C ,30^{o} C ,35^{o} C ,40^{o} C)

ns: number of drops of solvent

ds : density of solvent at temperatures (25^{o} C ,30^{o} C ,35^{o} C ,40^{o} C)

ɣs: surface tension of solvent

ɣw: surface tension of water at different temperatures

o $liquid\ density\ (ds) = \frac{wt.of\ solvent}{wt.of\ water\ \times density\ of\ water}$

4.2.4 Determination of Critical Micelle Concentration:

The conductivity data obtained was used to calculate CMC values by plotting the graph between conductivity and increasing wetting agent concentration. The slight change in the graph shows the CMC of lipase arrangement. Dual digressions were made and point of interaction between these dual straight lines was obtained as CMC values.

4.2.5 Thermodynamic Parameters Calculated:

The following equations were used to calculate the parameters of thermodynamics i.e ΔS,ΔH and ΔG:

- $\Delta H^0m = -RT^2\,(2-\alpha)\,(d\ lnX\ CMC)\ /\ dt$
- $\Delta S^0m = (\Delta H°m - \Delta G°m)\ /\ T$
- $\Delta G^0m = RT\,(2-\alpha)\,(lnX\ CMC)$

α denotes degree of ionization of surfactant

i. ΔG^{o} m : standard Gibbs free energy of micellization.

ii. ΔH^{o} m : standard Enthalpy for change of micellization.

iii. ΔS^{o} m : standard Entropy change of micellization.

4.2.6 Determination of Biological Oxygen Demand:

The Biological Oxygen Demand is proportion of amount of rot of natural issue present in water. The test squander water from clothing is gathered on Day 1 and its DO is estimated. Six additional examples containing 1% lipase with various convergences of CTAB, Tween-20 and SDS is added to tumbler flasks holding the clothing water. These bottles are kept in incubation for 5 days and on the fifth day the DO is taken and results are assessed.

4.2.7 Determination of Chemical Oxygen Demand:

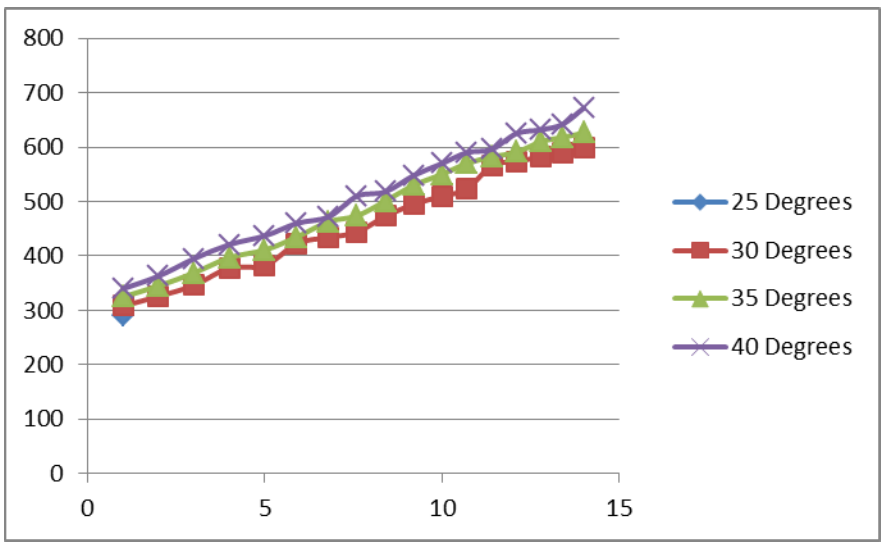
To determine COD 10 ml of Potassium dichromate(0.25N) is taken and 20 mL sample solution is added to it and with that 30mL of Silver sulphate sulphuric acid and 1mL of mercuric sulphate is added to reaction vessel. This reaction vessel is attached to digestion block which has to attain 120^{o} C temperature Reflux content for 2 hours.The content is then cooled and added to a 200mL flask and 80mL of distilled water is added to the flask. Titration is done for excess potassium dichromate using 4-5 drops of ferroin indicator. There is a change in colour from yellow to red brown .

After this readings are noted.

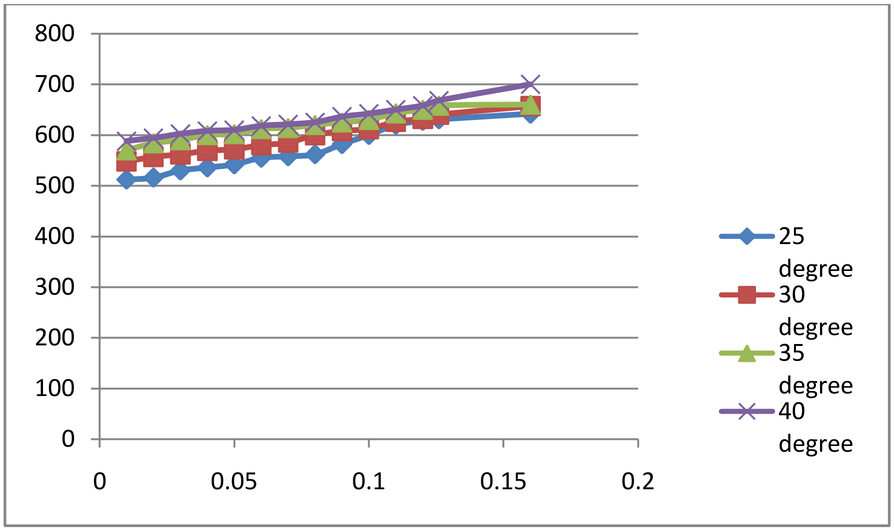
5.1 RESULTS AND DISCUSSION

5.1.1 Conductivity:

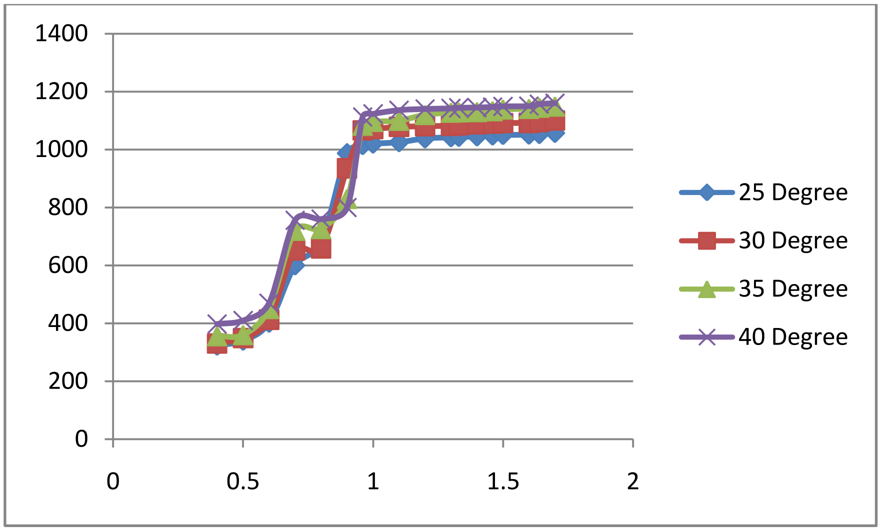
The particular conductance estimations of a number of wetting agents i.e CTAB, Tween-20 and SDS in nearness of 1% lipase was estimated and were then used to decide CMC values for every one of thewetting agents i.e CTAB, Tween-20 and SDS the conductivity changes can be seen from Plot 1-3. These estimations of CMC were utilized to compute the thermodynamic parameters .The estimations of CMC that were resolved tentatively of the wetting agents CTAB, Tween-20 and SDS in nearness of 1% lipase apparently fluctuated with various temperatures.



Plot 1 : SpecificConductivity vs Concentration of SDS in 1% Lipase



Plot 2: Specific conductance vs Tween -20 concentration in 1% lipase



Plot 3: Specific Conductance vs CTAB concentration in 1% lipase

5.1.2 Thermodynamic Parameters

The thermodynamic parameters were calculated using the CMC values for different surfactants for 1% lipase. The values of CMC for The ΔH°m values were positive that means that process was endothermic in nature.The values fluctuated at different temperatures and with changing concentrations of surfactants in presence of lipase. The ΔG°m were negative in all cases of different surfactants and the ΔS°m values were positive.

(i) SDS-Lipase

Table 3: Thermodynamic parameters for SDS-Lipase

TEMPERATURE	CMC	ΔH	ΔG	ΔS
298	0.0064	21.63267	-22.4706	0.147998
303	0.0079	22.36469	-22.3173	0.147465
308	0.0061	23.10889	-23.3476	0.150833
313	0.0084	23.86527	-22.8941	0.149391

(ii) Tween20 – Lipase

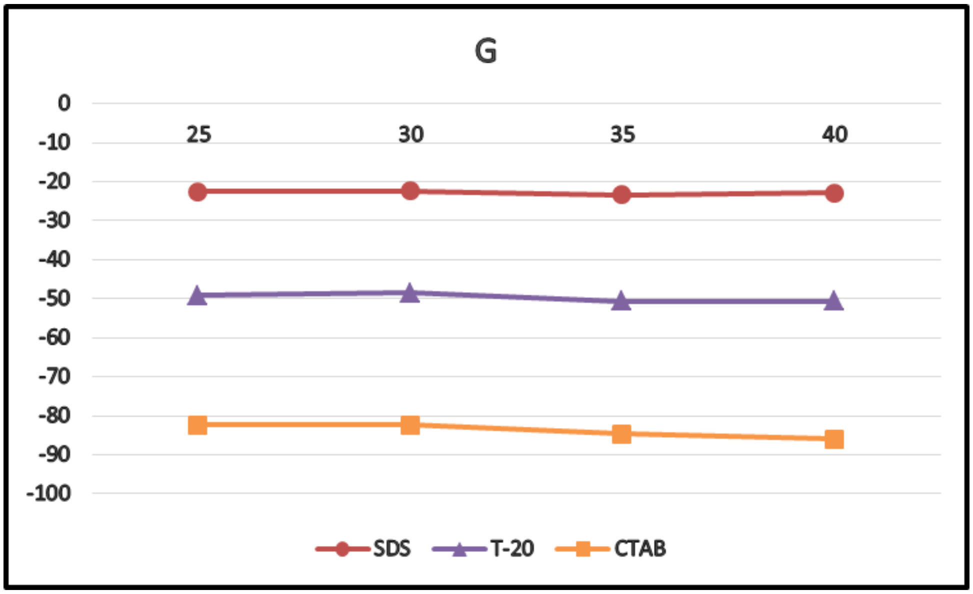
Table 4: Thermodynamics Parameters for Tween20- Lipase

TEMPERATURE	CMC	ΔH	ΔG	ΔS
298	0.0012	25.61958	-26.6178	0.175293
303	0.00174	26.48651	-26.1284	0.173647
308	0.00134	27.36787	-27.2284	0.177261
313	0.00132	28.26365	-27.7096	0.178828

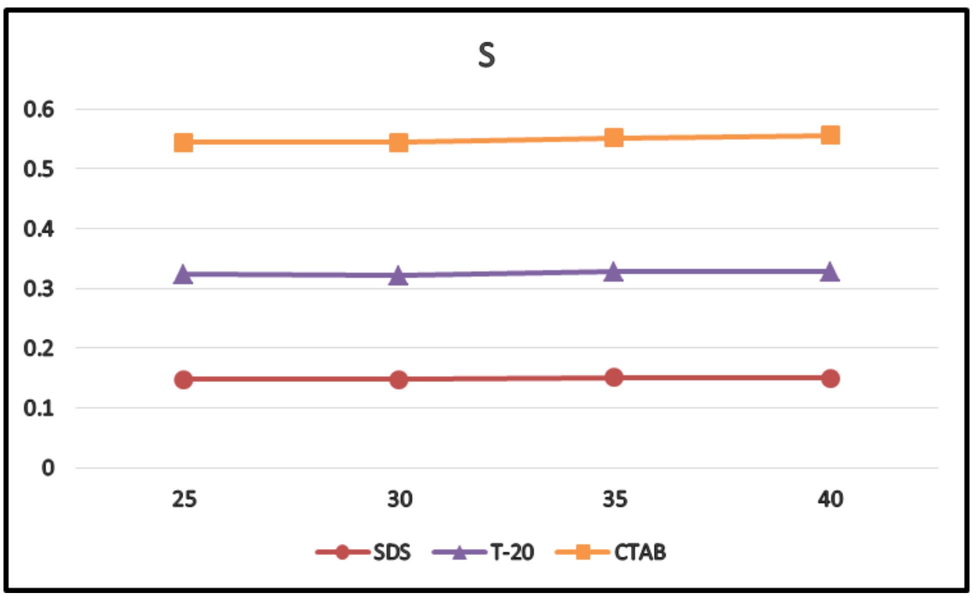
(iii) CTAB-Lipase

Table 5: Thermodynamic Parameters for CTAB-Lipase

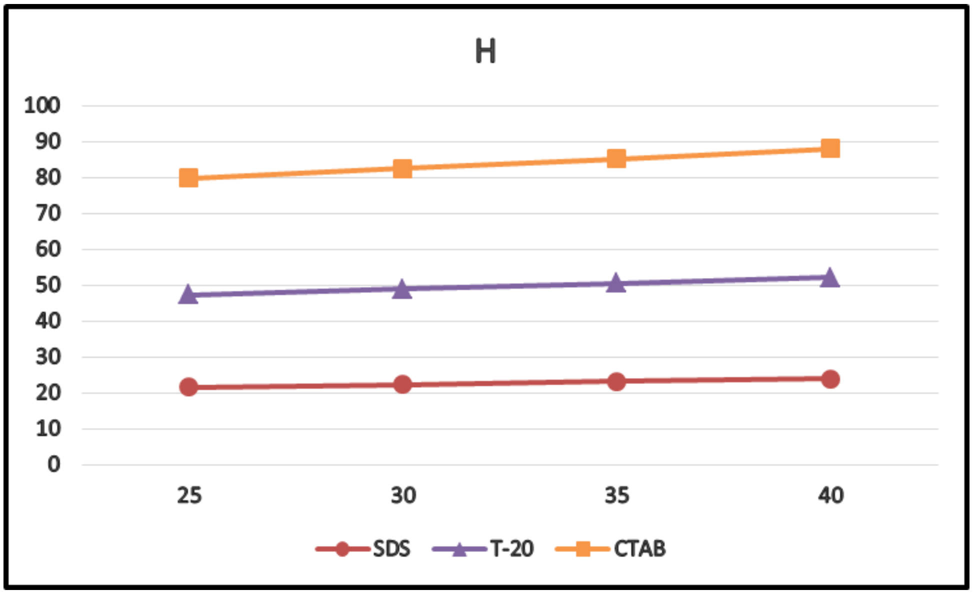
temp	cmc	Xcmc	T^2	LnXcmc	dLnXcmc	DhoM	Dgo	Dso
298	0.00008	1.44E-06	88804	-13.4515	-0.044	32.48592	-33.3271	0.220849
303	0.00008	1.44E-06	91809	-13.4515	-0.044	33.5852	-33.8863	0.222678
308	0.00009	1.62E-06	94864	-13.3337	-0.044	34.70277	-34.1439	0.223528
313	0.00007	1.26E-06	97969	-13.5851	-0.044	35.83863	-35.3521	0.227447



Plot 4: The plot of ΔG at 4 different temperatures



Plot 5: The plot of ΔS at 4 different temperatures



Plot 6: The plot of ΔH at 4 different temperatures

5.1.3 Surface Tension Studies:

The surface strain of all surfactants at a number of fixation and at various heats (25, 30, 35 and 40^{o} C) were assessed. To decide the surface tension of test, taking 0.25 g of Lipase in 25ml refined water to make stock arrangement and afterward including a little centralization of Tween 20 and stalagometric strategy (checking number of drops) was utilized and the thickness of arrangement of catalyst and surfactant is determined with assistance of explicit gravity bottle and weighing balance (they were done independently for various heats i.e (25^{o} C, 30^{o} C, 35^{o} C, 40^{o} C) .

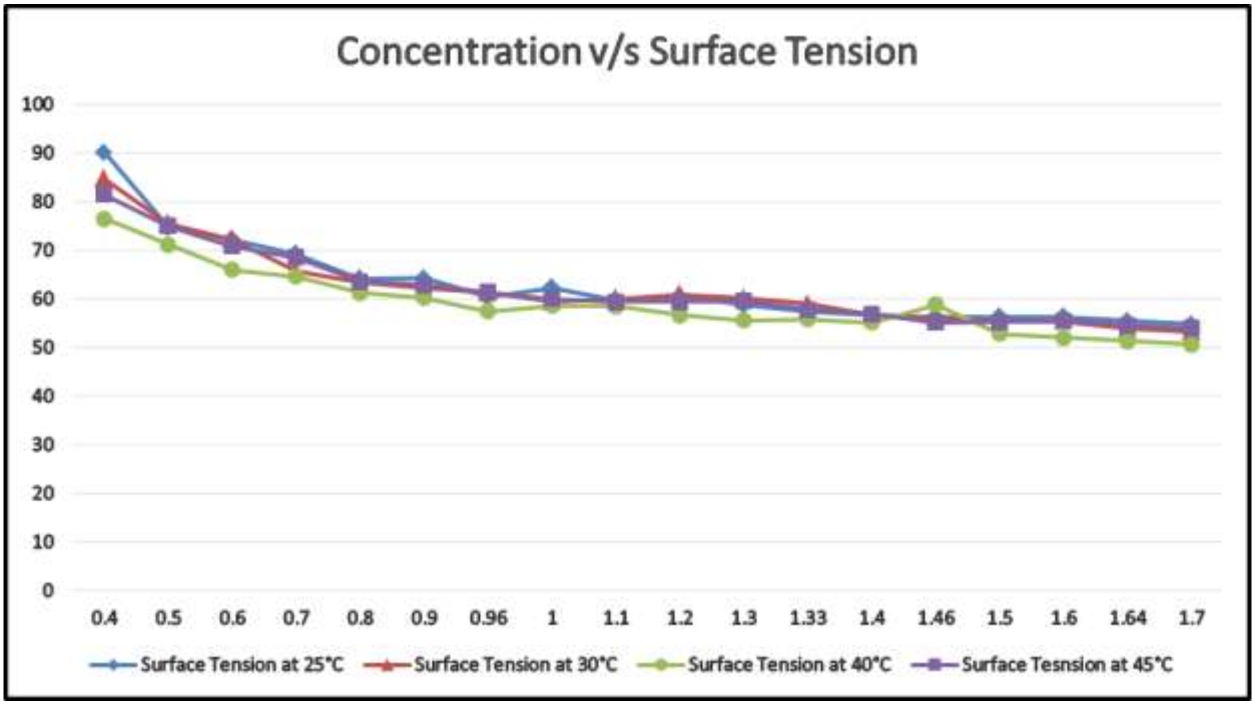
The number of drops was counted and done for the surfactants at different temperatures. The density for each sample is calculated which is further used in calculating the surface tension. The values of density of water at particular temperatures was also used in calculations.

The variation in surface tension in different surfactants is shown in Plot 7-9.

Surface Tension of CTAB

Table 6: The values of Surface Tension in presence of CTAB at 4 diverse temperatures

Concentration	**ɣs**			
	25⁰ C	**30⁰ C**	**35⁰ C**	**40⁰ C**
0.4	90.11	84.65	76.50	81.46
0.5	74.69	75.46	71.16	75.06
0.6	72.25	72.27	66.02	70.82
0.7	69.21	65.77	64.64	68.53
0.8	64.22	63.34	61.27	63.61
0.9	64.24	62.21	60.2	62.84
0.96	60.39	61.29	57.52	61.37
1	62.33	59.42	58.58	59.99
1.1	59.77	60.01	58.51	59.45
1.2	60.61	60.90	56.61	59.47
1.3	58.73	60.12	55.61	59.57
1.33	57.46	58.99	55.91	57.87
1.4	56.82	56.66	55.21	56.85
1.46	56.32	56.07	58.84	55.2
1.5	56.35	55.34	52.86	55.33
1.6	56.36	55.36	52.1	55.56
1.64	55.54	53.84	51.38	54.63
1.7	54.87	53.16	50.65	53.97

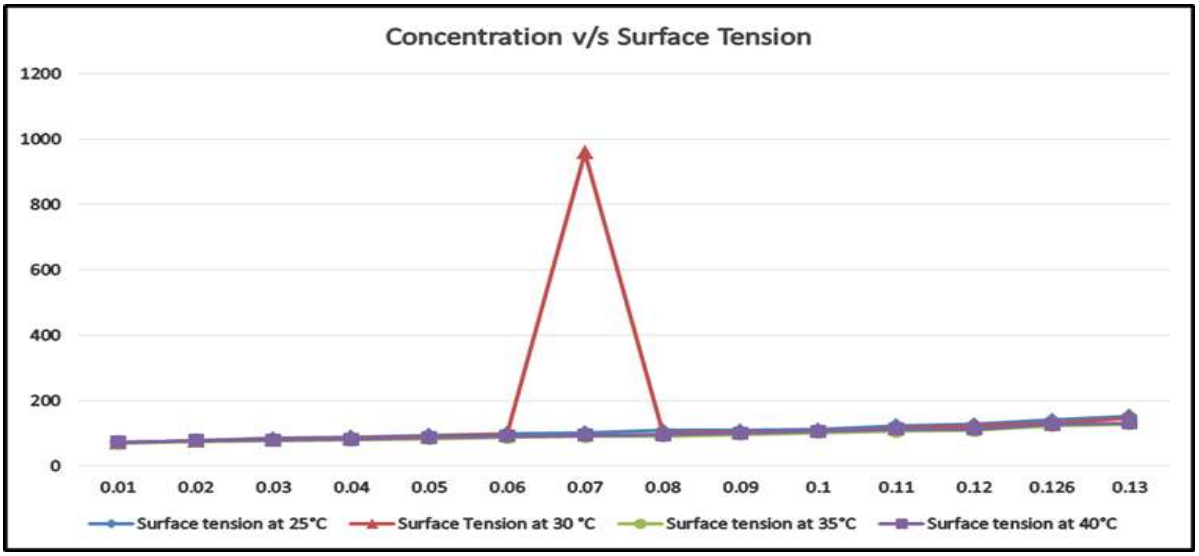


Plot 7: The plot for the different concentrations of CTAB vs Surface Tension at 4 different temperatures

Surface Tension of Tween -20

Table 7: The values of Surface Tension in presence of Tween-20 at 4 diverse temperatures

Tween-20 conc.	ɣs			
	25⁰	**30⁰**	**35⁰**	**40⁰**
0.01	71.925	71.894	68.870	72.915
0.02	78.041	77.157	75.690	77.927
0.03	84.481	82.140	77.535	79.844
0.04	88.72	84.205	79.258	81.543
0.05	93.821	90.596	83.041	87.024
0.06	99.183	95.511	87.148	91.132
0.07	102.089	958.232	89.421	93.361
0.08	108.331	100.926	91.775	95.679
0.09	108.397	103.929	96.797	100.648
0.10	111.830	107.05	102.405	105.587
0.11	123.448	113.86	105.484	112.284
0.12	127.90	121.473	108.749	112.382
0.126	140.652	130.260	123.857	126.777
0.13	152.435	145.99	128.362	131.292

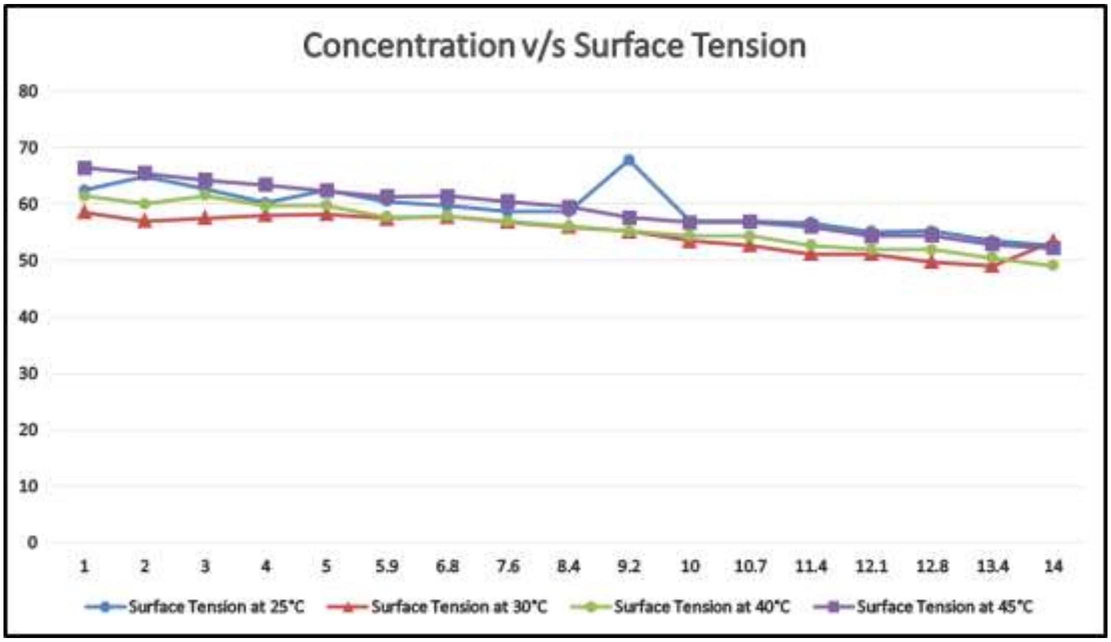


Plot 8: The plot for different concentrations of T-20 vs Surface Tension at 4 different temperatures

Surface Tension of SDS

Table 8: The Surface Tension of SDS of different concentration at different temperatures

Concentration	**ɣs**			
	25⁰	**30⁰**	**35⁰**	**40⁰**
1	62.435	58.560	61.387	66.382
2	64.932	57.023	60.029	65.417
3	62.610	57.533	61.477	64.279
4	60.142	58.013	59.670	63.387
5	62.485	58.179	59.711	62.352
5.9	60.477	57.421	57.711	61.338
6.8	59.688	57.748	57.856	61.402
7.6	58.639	56.847	56.914	60.420
8.4	58.664	55.947	56.022	59.471
9.2	67.752	55.207	55.141	57.604
10	56.878	53.423	54.289	56.730
10.7	56.902	52.643	54.338	56.795
11.4	56.614	51.095	52.676	55.966
12.1	55.119	51.139	51.895	54.321
12.8	55.202	49.738	51.930	54.371
13.4	53.467	49.046	50.397	52.838
14	52.68	53.388	49.030	52.118



Plot 9: The plot for different concentrations of SDS vs Surface Tension at different temperatures

5.1.3 Biological Oxygen Demand:

After surface tension the natural oxygen request is resolved of lipase with surfactants. This is performed to watch that at which grouping of catalyst and surfactant is necessitated that the cleanser is proficient in cleaning. The various groupings of SDS (7.9, 8.4 µL), CTAB (1.32, 1.34µL) and Tween 20 (0.082, 0.099µL) were taken. The container with least DO can be utilized for detergent formulation.

Bottle Number	**D.O (mg/L)** **Day 1**	**D.O (mg/L)** **Day 5**	**BOD**
1	8.40	0.49	74.6
2	8.34	0.54	78
3	8.30	0.36	79.4
4	8.15	0.38	77.7
5	8.39	0.85	75.4
6	8.16	0.31	78.5

5.1.4 Chemical Oxygen Demand

Sample Bottle No	**COD Value**	**COD= COD value ×Dilution Factor**
1	23.7	277
2	23.6	236
3	24.18	241
4	24.1	241
5	22.70	227
6	23.9	239

5.2 CONCLUSION:

The fundamental goal of this study was to detail a green-cleanser that is proficient and furthermore is degradable, non-poisonous and doesn't influence nature. The investigation of examination of physicochemical properties, conductivity, thermodynamic parameters is finished utilizing a number of wetting agents i.e CTAB, Tween-20 and SDS in shifting focuses in nearness of lipase. Further examinations like surface pressure study, BOD were additionally directed to help bolster the investigation. The natural oxygen request considers helped in finding the ideal convergence of surfactant with 1% lipase for definition of cleanser. Along these lines, the convergence of surfactant with 1% lipase can be utilized to detail a cleanser having greater degradable ability without bargaining with washing proficiency.

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