Comparative Interaction Study of Enzymes and Surfactants for Potential Detergent Formulation

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CERTIFICATE OF ORIGINALITY

This is to certify that the thesis entitled: **"Comparative Interaction Study Of Enzymes And Surfactants For Potential Detergent Formulation"** submitted by **Ashwina Singh** and **Akshay Sharma** in partial fulfillment of the requirements for the award of degree of Bachelors of Technology in Biotechnology, Jaypee University of InformationTechnology, Solan, has been carried out under the supervision of Dr. Poonam Sharma. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

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(Akshay Sharma and Ashwina Sngh)

ABSTRACT

Detergent enzymes are environment friendly and are common in advanced countries. Enzyme based detergents have been used for remodeling the cleaning efficiency of detergents for many years. Despite of the actuality that the detergent business is that the largest single galleria for enzymes total barter, particulars of the enzymes used and therefore the design within which these enzymes are used, have seldom been printed. This paper investigates the physico-chemical properties to obtain the critical micellar concentration (CMC), the thermodynamic parameters i.e the values of ΔH°m, ΔG°m, ΔS°m of surfactants i.e anionic surfactant sodium dodecyl sulphate (SDS), cationic surfactant cetyl trimethylammonium bromide (CTAB) and non-ionic surfactant (TWEEN 20) in presence of α-amylase, from *Aspergillus oryzae* and enzyme from *Aspergillus niger* at completely different temperatures 25, 30, 35, 40ºc kinetic studies i.e the Km and Vmax values that are measured by Michaelis-Menten mechanics, surface tension studies and the degradation studies that involves the (BOD) biological oxygen demand to conduct a comparative analysis for potential detergent formulation.

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Akshay Sharma Ashwina Singh Dr. Poonam Sharma

(Signature of students) (Signature of supervisor)

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CHAPTER 1

NTRODUCTION

Detergent containing enzymes are referred to as 'green detergents' attributable to their biodegradability, low toxicity, non-corrosiveness, environmental friendliness, increased improvement properties moreover as inflated potency and stability in numerous formulations [1, 2]. Detergent composition usually includes six teams of substances: surfactants, builders, enzymes, bleaching agents, fillers and alternative minor additives like optical brighteners [3, 4].

The use of enzymes in detergents permits lower temperatures to be used and shorter periods of agitation are required, usually once a preliminary amount of soaking. In general, catalyst detergents take away super molecule from garments fouled with blood, milk, sweat, grass, etc. way more effectively than non-enzyme detergents [5].

With respect to the enzyme, several surfactants that interplay with proteins and their structure and alter the secondary and tertiary structures of proteins due to different electrical charges and different hydrophobic groups and hydrophilic groups [6]. Specially, several enzymes are unstable in solutions of anionic surfactants like Na ether salt, sodium dodecyl sulfate (SDS), additionally, the formation of micelles may also have an effect on catalyst dynamics [7].

Surfactants, or active agents, are chemicals that cut back the physical phenomenon of oil and water. In detergents, surfactants facilitate dirt to drop out and keep out of vesture or alternative things being cleansed [8]. Enzymes are biological molecules that turn (speed up) chemical reactions. Enzymes are specific they can solely work on explicit molecules [9].

Enzymes are employed in soap as a result of stains are fabricated from differing molecules, a spread of enzymes are required to interrupt them down. Proteases break down proteins, therefore are sensible for blood, egg, gravy, and alternative super molecule stains [10,11]. Amylases break down starches, and lipases break down fats and

grease. Laundry powders typically solely contain one style of catalyst, although some have 2 or all 3 [12].

Surfactants used:

1) Cationic surfactant

The dissociated substance produces a charged hydrophobic particle. CTAB cetyl trimethyl ammonia bromide (364.46 g/mol) is that the best illustrious example.

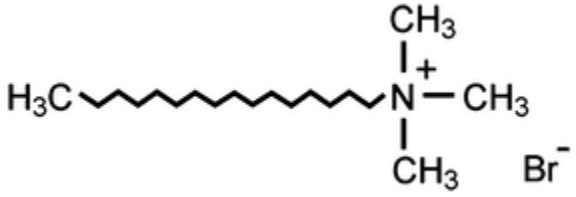

Fig1.1 Stuctural illustration of CTAB

2) Non-ionic surfactant

Any of a category of artificial detergents (as long-chain ether derivatives or esters of alcohols or phenols) that are neither anionic nor cationic however they turn out electrically neutral mixture particles in answer. TWEEN twenty (522.676 g/ mol) and Triton X- one hundred (647 g/mol) are the examples [13].

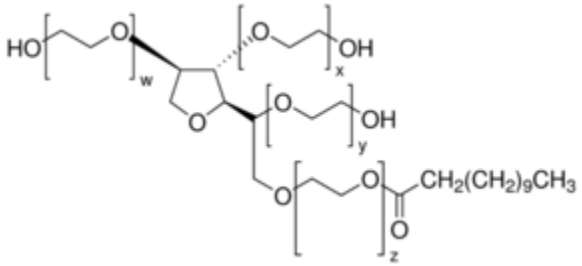

Fig. 1.2 Stuctural illustration of TWEEN 20

3) Anionic surfactant

A substance that once dissolved contributes a hydrophobic particle that carries a negative charge to the answer. (SDS) Sodium dodecyl sulfate (288.38 g/mol) is an associate degree example [14].

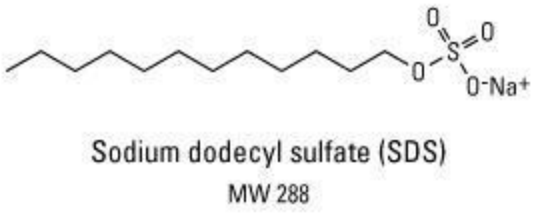

Fig. 1.3 Stuctural illustration of SDS

Micellization:

When surfactant is added to water, the surfactant molecules get dissolved in water. Further on increasing the concentration of surfactant in water, aggregates of surfactant molecules are formed and the phenomenon of formation of aggregates is known as micellization [15].

Micelles are the nano - sized stable aggregates of wetting agent molecules, that kind impromptu in wetting agent solutions. When the surfactant concentration is increased above the critical micelle concentration (CMC) the reaction of micelles within the surfactant becomes nonetheless vital [16].

Above the critical micelle concentration the micelle are readily formed thus enhancing the cleaning property of the surfactant.The head is hydrophilic and tends to contact with water whereas the tails are hydrophobic thus located away from the water phase.

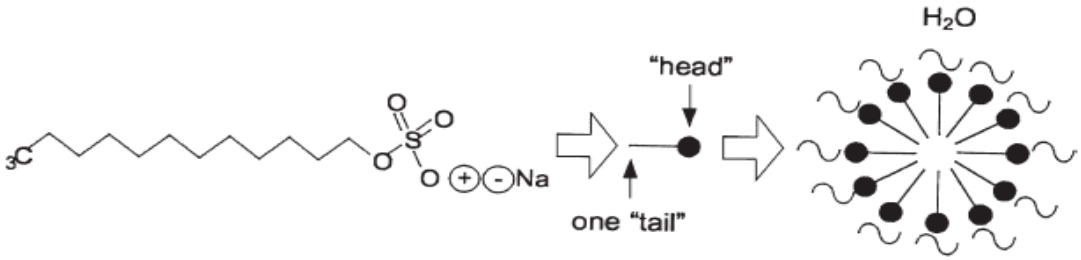

Fig. 1.4 Stuctural representation of micelles formation.

Enzymes used:

1) Amylase is associate degree catalyst that catalyses the reaction of starch into sugars. Enzyme is gift within the spittle of humans and a few alternative mammals, wherever it begins the chemical action of digestion. Amylases in detergents breaks down starches and thereby simple stain removal. Alpha-amylase from genus *Aspergillus oryzae* by Himedia was employed in the experiment [17].

2) Lipase is one in every of our most significant biological process enzymes free chiefly by the exocrine gland into the little internal organ to assist the activity and absorb fats. Lipases are a taxonomic group of esterases and it chemical process reaction of fats (lipids). Lipases in detergents helps in removing fats and grease stains. Enzyme from genus *Aspergillus niger* by Loba Chemie.

In the present thesis, different physico- chemical studies including comparative interaction study between amylase, lipase and different surfactants have been performed to locate a preferred concentration witch would be helpful for detergent formulation to increase biodegradability without compromising with its cleaning efficiency.

CHAPTER 2

LITERATURE REVIEW

The relevant representation studies of interaction between enzymes and surfactants have been summarized in this section. A great deal of strenuous efforts have been reported in literature to analyze interactions between enzymes and different classes of surfactants.

Hendrik Hellmuth *et al*. [1] conducted an investigation of the interaction of surfactants and enzymes below detergent application conditions so as to grasp the influence of individual ingredients and to optimize detergent performance. They were able to show that for a given proteolytic accelerator enzyme, individual surfactants during a constant detergent matrix have a major impact on relevant stability and performance parameter. Whereas sure anionic surfactants like e. g. linear organic compound salt show robust proteinase inactivation, nonionic surfactants did solely show slight inactivation over time. On the opposite hand, chemical action performance of proteinase on take a look at stains was most driven by fatty alcohol ether sulphate. Information concerning the impact of individual wetters on proteases can change the most effective alternative of ingredients for mixed surfactant systems with optimized accelerator performance and stability.

Hans Sejr Olsen *et al*. [2] studied the role of enzymes in fashionable utility. Enzymes have effectively aided the event and improvement of contemporary unit and industrial detergents. The key categories of detergent enzymes—proteases, lipases, amylases, and cellulases—each give specific edges for application in laundry and automatic lavation. Traditionally, proteases were 1st to be used extensively in laundry detergents. Additionally to raising the amount of cleansing, they need additionally provided environmental edges by reducing energy consumption through shorter laundry times, lower laundry temperatures, and reduced water consumption. These days proteases are joined by lipases and amylases in up detergent efficaciousness particularly for unit lavation at lower temperatures and, in industrial cleansing operations, at lower hydrogen ion concentration levels. Cellulases contribute to overall

material care by rejuvenating or maintaining the new look of washed clothes. Enzymes are created by fermentation technologies that utilize renewable resources.

YU Yangxin *et al*. [3] investigated that surfactants and builders are the 2 most vital ingredients in laundry, unit and personal-care cleansing product. They play a key role in laundry processes. The event of assorted surfactants (e.g., anionic, nonionic, cationic, zwitter ionic, and polymer surfactants) and builders (inorganic, organic and chemical compound builders) utilized in the detergent compositions are reviewed and their utility performance and biodegradability are mentioned. Within the future, the event of the surfactants and builders utilized in detergent compositions ought to be supported economic and environmental issues. The utilization of the eco-friendly surfactants and builders derived from cheap renewable resources (e.g., alkyl radical polyglucosides and bio-based polyesters) in detergent compositions is that the developing trends in detergent trade.

Masako Sato *et al*. [4] investigated the impact of builders on the steadiness of protease enzyme activity was studied in an endeavor to spot superior builders that are soluble in water and compatible with enzymes developed into serious duty laundry powders. numerous poly(styrenesulfonate-methacrylate) copolymers, polyacrylate and tripolyphosphate anionic builders, in addition as numerous poly(vinylalcohol-vinylacetate) nonionic copolymers, specifically PVAs, were used. mineral 4A was additionally used as a typical nonphosphate particulate builder within the detergents. The proteinase used is frombacillus stearothermophilus. The atomic number 20 content was resolute to be sixteen.7 mole/mole of proteinase by atomic spectrophotometry. In binary systems composed of a set concentration of ten U/mL proteinase and varied concentrations of compound, builder or surfacant, it had been found that compounds having the larger clotting factor binding capability (C.B.C.) lowered the relative activity of proteolytic accelerator enzyme. The activity of proteolytic accelerator enzyme alone was lowered concerning 2 hundredth by addition of zero.02% metallic element dodecylbenzene salt (DBS). The anionic builders further to the binary number system of fastened ten U/mL proteinase and zero.02% DBS cut back the proteolytic accelerator enzyme activity in proportion to the

magnitude of their C.B.C. Addition of anionic builders additional lowered the proteolytic accelerator enzyme activity. The nonionic builders and therefore the nonionic wetter will enhance the proteolytic accelerator enzyme activity by protection of proteinase against the matter, DBS. It's sure that atomic number 20 atoms contained within the proteolytic accelerator should play a vital role for the proteinase enzyme activity and its stability. Atomic number 20 atoms should have a good influence on the formation of protease-substrate advanced, protease-compound advanced and substrate-compound advanced, as a result of the proteinase, macromolecule substrate and chemical compound would all be charged in basic solutions. Builders for enzyme-containing detergents ought to be made to be insensitive to clotting factor.

L. Kravetz *et al*. [5] studied the impact of wetter structure on accelerator stability in serious duty laundry liquids was investigated. Surfactants studied were alcohol ethoxylates and anionic surfactants having variable hydrophobic and hydrophilic sorts and chain lengths. Enzymes used were proteases and amylases. The results showed these enzymes were significantly additional stable once developed into laundry liquids containing alcohol ethoxylates and ethoxysulfates than once developed with alcohol sulfates and surfactants containing salt teams like linear organic compound sulfonates and alpha alkene sulfonates. Accelerator stabilizer systems were solely part effective in reducing the enzyme deactivating influence of sulfonate-containing surfactants.

Jim Lalonde *et al*. [8] found that anionic surfactants like linear alkyl radical benzene salt (LAS) will solubilize proteases during a well non aqueous surroundings while not loss of chemical action activity. Moreover, in mixtures of anionic and nonionic surfactants with a moderate quantity of water (water but thirty wt%), controlled levels of LAS and water solubilize proteases; nonetheless, in these targeted wetter mixtures, enzymes maintain their activity for extended periods. Experimental style techniques are accustomed delineate the link between proteinase stability and therefore the water, hydrogen ion concentration and anionic wetter levels in these wetter concentrates. Because the total of water and LAS levels is inflated, most accelerator stability is discovered, once that stability falls off. At low tide and LAS levels (sum

of each & lt 20%), proteinase solubility is low, whereas at high levels of water and LAS (sum of LAS and water & gt 45%), denaturation predominates. in addition, we've got developed a replacement and straightforward technique to predict proteinase stability by that an artificial amide is employed to live proteinase activity directly within the wetter concentrate. From the applying of this new technique to our system and to business detergent formulations, it's apparent that water facilitates the loss of activity of proteases in wetter concentrates by increasing the speed of lysis.

Grbavcic S *et al*. [10] studied an autochthonic Pseudomonas aeruginosa strain for enzyme and proteinase activities for his or her potential application in detergents. Created enzymes were investigated so as to assess their compatibility with many surfactants, oxidizing agents and business detergents. The crude enzyme looked as if it would retain high activity and stability within the presence of many surfactants and oxidizing agents and it had been immune to chemical process. Lutensol XP eighty and Triton X-100 powerfully activated the enzyme for a protracted amount (up to forty and half-hour against the management once one h) whereas the proteinase activity was increased by the addition of Triton WR1339 and Tween eighty. The laundry performance of the investigated surfactants was considerably improved with the addition of the crude accelerator preparation. Studies were additional undertaken to enhance enzymes production. The optimisation of fermentation conditions diode to AN 8-fold increase of enzyme production, whereas the assembly of proteinase was increased by hr.

A Haddar *et al*. [11] studied that 2 detergent stable basic serine-proteases (BM1 and BM2) from Bacilli mojavensis A21 were pure. The molecular weights of BM1 and BM2 enzymes determined by SDS–PAGE were or so 29000 prosecuting officer and fifteen, 500 Da, severally. The optimum hydrogen ion concentration values of BM1 and BM2 proteases were shown to be eight.0–10.0 and 10.0, severally. Each enzymes exhibited greatest activity at sixty °C, victimization casein as a substrate.

The N-terminal aminoalkanoic acid sequences of BM1 and BM2 proteases were AQSVPYGISQIKA and AIPDQAATTLL, severally. Each proteases showed high stability

towards non-ionic surfactants. The enzymes were found to be comparatively stable towards oxidizing agents. Additionally, each enzymes showed glorious stability and compatibility with a large vary of economic liquid and solid detergents. These properties and therefore the high activity in high basic hydrogen ion concentration create these proteases a perfect alternative for application in detergent formulations.

A Tanaka *et al.* [12] studied kinetic action within the reaction of amylose with 2 microorganism α-amylases from Bacilli amyl liquefacients and B. licheniformis were studied for effects of metallic element dodecyl sulphate (SDS) on those 2 microorganism species. Sigmoid mechanics were seen with inflated concentration of further SDS in chemical process rates of each amylases within the gift study; inflated rates were utilized in the vary below the crucial particle concentration (CMC) and so significantly remittent higher than the CMC. it had been seen that the chemical process rate of the α-amylase from B. amyloliquefaciens was additional alert to the further wetter than that of the α-amylase from B. licheniformis. The formation of the enzyme-substrate advanced was attributable to the diluted SDS at concentrations below the CMC, Additionally the hydrolytic contact action of each enzymes was allegedly accelerated. The thermodynamiccriterion for the E-S advanced formation Affirmed that the probable heat content and entropy changes of each amylases were inflated by the addition of the diluted SDS. In the end, it had been seen that the larger increase in entropy changes for E-S advanced formation was attributable to increase in hydrolytic rate by addition of SDS instead of in heat content changes of that E-S advanced.

Steen Arnesen *et al*. [13] discovered that thermomyces lanuginosus a thermophilic plant life was cultivated in shake flasks for up to a hundred and twenty h with low mass dextran as carbon supply supplemented with either Triton X-100 or Tween eighty. Once Tween eighty was further to the expansion medium there was a pair of.7-fold increase in most α-amylase activity as compared with controls. The α-amylase production wasn't plagued by addition of Triton X-100. within the presence of Tween eighty, the α-amylase secretion got aroused from the terribly third day of cultivation whereas there was increase in general macromolecule secretion once solely

twenty four h of cultivation. By high concentrations of Tween eighty the number of biomass created additionally inflated slightly however remittent by the addition of Triton X-100. Quite three-fold increase was found in most total extracellular macromolecule with increasing concentrations of Tween eighty within the medium. Neither the hyphal diameter nor the hyphal growth unit length (G) were plagued by Tween eighty. The degree of glycosylation of the α-amylase wasn't altered by addition of Tween80. Once Tween eighty was further it did seem to offer a general stimulation of macromolecule secretion.

Hoshino E *et al*. [17] studied that some enzymes once developed with non-ionic surfactants are additional stable as compared to anionic surfactants. A study was conducted to check the impact of a nonionic wetter, polyoxyethylene mono-N-dodecyl ether, (Brij 35; variety of units of gas compound moieties, 23) on the kinetic behaviour of reaction of amylopectin with Bacilli amyloliquefaciens α-amylase (BAA at a temperature of 25°C and a hydrogen ion concentration of seven.0. By the addition of the nonionic wetter higher than its crucial particle concentration the hydrolytic rate was hastened. Linear relationships were seen in Lineweaver-Burk plots for the protein reaction within the absence and presence of the nonionic wetter at zero.5 to 2.5% (wt/vol), and therefore the kinetic parameters, km and kcat were obtained. Brij thirty five was accustomed increase the worth of kcat , whereas the km price was or so constant. The rise in apparent hydrolytic rate was attributable to kcat. Examination by a physical phenomenon mensuration was done to indicate the interaction of amylopectin with wetter, additionally with the result it had been confirmed the corresponding attachment between the substrate and therefore the wetter. Attributable to presence of essential amino acid in BAA a visible light analysis was done witch projected that BAA guaranteed to non-ionic micelles. The rise in kcat disclosed that hydrolytic contact action at the micellar pseudophase was additional adequate than that at the liquid pseudophase. The effective removal of food stains was attributable to the improvement of chemical process rate that was earned once BAA was further with Brij thirty five during a detergent laundry take a look at.

YAN Sangtian *et al*. [19] conducted a study to review the consequences of ionic surfactants on microorganism luciferase. For this dodecyltrimethyl ammonia bromide (DTAB) an ion wetter and metallic element dodecylsulfate (SDS) an anionic wetter were chosen, so as to own a comparison with microorganism luciferase, α-amylase was used. The enzymes were treated with the surfactants, then the chemical process properties of each the enzymes were assayed, and therefore the alteration of the macromolecule structure was analyzed usingfluorescence qualitative analysis and circular pleochroism (CD). in keeping with the results once the DTAB concentration was low, the ion wetter DTAB increased the protein activities of each the enzymes, microorganism luciferase and α-amylase, whereas the anionic wetter SDS failed to alter the protein activity in the least. The most interaction between the charged surface of the proteins and DTAB was ionic interaction; this might have altered the surroundings for the accelerator to figure once the molar magnitude relation of DTAB/enzyme was low. However, at high concentration of ion wetter, the ionic interaction and therefore the hydrophobic interaction may destroy the secondary in addition because the tertiary structures of the proteins, thereby resulting in the loss of protein activities.

Jian Zhang *et al*. [22] investigated the consequences of surfactants on proteinase with and while not stabilizer boracic acid. From the structural options of surfactants, we tend to mentioned the potential mechanism between surfactants and proteinase. Surfactants studied were anionic surfactants having variable hydrophilic cluster and nonionic surfactants having variable hydrophobic sorts and chain length. The results showed that anionic wetter linear alkyl radical benzine salt (LAS) has the most important influence on proteinase, attributable to benzol in its structure; nonionic wetter fatty alcohol polyoxyethylene (AEO) with additional gas compound shows larger impact on proteinase; carbon chain length in alkyl radical polyglucosides (APGs) has less influence on protease. Understanding the reaction mechanism of surfactants and proteinase is important for several trade uses. Particularly the results give references for the applying of proteinase in laundry formulation.

2.2 Objective

- To investigate the physico-chemical properties to obtain the critical micellar concentration (CMC) and thermodynamic properties of surfactants i.e Sodium dodecyl sulfate (SDS), cetyl trimethylammonium broide (CTAB), TWEEN 20 in presence of enzymes i.e amylase and lipase at different temperatures 25, 30, 35, 40ºC.

- Comparative interaction study of physico-chemical properties of surfactants i.e Sodium dodecyl sulfate (SDS), cetyl trimethylammonium broide (CTAB), TWEEN 20 in presence of different enzymes i.e amylase and lipase and their degradation studies to check the biodegradability of conc. of surfactants and enzymes which can be further used for detergent formulation without compromising with the cleaning efficiency of the detergent.

CHAPTER 3

EXPERIMENTAL

3.1 Material

Sodium dodecyl sulfate (SDS), Cetyl trimethyl ammonium bromide(C-TAB),Tween 20, α-amylase, from *Aspergillus oryzae*(1:2000 U, HI-MEDIA), lipase from *Aspergillus niger* (2500 U/ml).

3.2 Conductance measurements:

3.2.1 Specific Conductance (SC) is a measure of how well water can conduct an electrical current. These ions, which come from the breakdown of compounds of water will conduct associate degree electrical current, physical phenomenon of conductivity will increase with increasing quantity and quality of ions. These ions, that come back from the breakdown of compounds, conduct electricity as a result of their becoming negatively charged once dissolved in water.

3.2.2 Critical micelle concentration (CMC) is defined as the concentration of detergents above which micelles are spontaneously formed. The CMC is very important in biology as a result of concentrations and on top of it addition to the detergents to form complexes with lipotropic proteins. Below this borderline, detergents simply partition into membranes while not solubilising membrane proteins.

CMCs vary with ionic strength and temperature. By increasing the ionic strength of the solution for ionic detergents reduces the CMC, however is comparatively unaffected by temperature but in case of non-ionic detergents the CMC is however unaffected by the ionic strength,but CMC will increase considerably with higher temperature.

3.2.3 Thermodynamic studies

$\Delta H°m = - RT^2 (2-\alpha) (d \ln Xcmc) / dt$

$\Delta S°m = (\Delta H°m - \Delta G°m) / T$

$\Delta G°m = RT (2-\alpha) (\ln Xcmc)$

α denotes the degree of ionization of surfactant , ΔG°m is the standard Gibbs free energy change of micellization. , ΔH°m is the standard Enthalpy for micellization., ΔS°m is the standard Entropy of micellization.

3.3 Kinetic studies:

3.3.1 Michaelis-Menten kinetics:

The Michaelis-Menten equation: defines associate degree initial-rate equation for a single-substrate non cooperative enzyme-catalyzed reaction relating the initial speed to the initial substrate concentration.

$$V_0 = \frac{V_{\max}[S]}{(K_M + [S])}$$

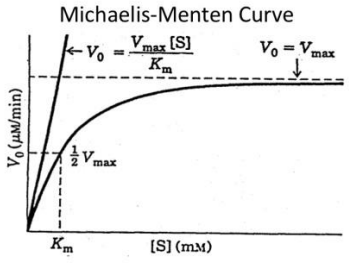

Fig. 3.1 Michaelis-Menten equation and its Graphical representation.

In this equation $\mathbf{V_0}$ is the velocity of the reaction, $\mathbf{V_{max}}$ is the maximal rate of the reaction, **[S]** is the concentration of the substrate, $\mathbf{K_m}$ is the Michaelis-Menten constant that shows the concentration of the substrate once the reaction speed is capable one half the greatest velocity for the reaction. It can also be thought of as a measure of how well a substrate complexes with a given enzyme, otherwise called its

binding affinity. An equation with a low K_m value indicates an outsized binding affinity, because the reaction can approach V_{max} more rapidly. An equation with a high K_m indicates that the enzyme does not bind expeditiously with the substrate, and V_{max} can solely be reached if the substrate concentration is high enough to saturate the enzyme.

3.3.2 Enzyme activity:

Enzyme activity refers to the overall catalytic properties of associate degree catalyst i.e enzyme, and standardized catalyst assay procedures to detect the amounts of specific enzymes in a given sample.

- Enzyme activity = $\frac{\text{amount of glucose released (mg/ml)} \times 1000}{\text{Volume of enzyme used} \times \text{incubation time} \times \text{mol. Wt of Glucose}}$

3.4 Surface tension :

The formation of the droplets that flows freely from the capillary, depends on the physical phenomenon of the liquid. This development is employed to work out the physical phenomenon using the number method i.e the stalagmometric technique (method of counting the no. of drops). Stalagmometer is a device created from a glass bulb with marked on top of and below the bulb indicators designating specific volume of liquid, complete capillary. To determine the surface tension of the sample i.e a liquid, fill the stalagmometer with the sample upto to upper marked point by sucking the sample into the stalgmometer through the rubber tube attached to the upper part of the stalgmometer and then permit the liquid to flow freely by slowly releasing the screw on the rubber tube attached to the stalgmometer to control the flow of the liquid. While the sample flows out of the stalgmometer count the number of drops for each sample. Now repeat the procedure with the samples at different temperatures (25, 30, 35, 40°C) and record the number of drops for each sample with varying temperatures.

$$\gamma s = \left(\frac{ds.\ nw}{dw\ .ns}\right) \times \gamma w\ dyne/cm$$

Density of liquid (ds) = (wt. of solvent/wt. of water) × Density of water

Wherein nw is the no. of drops of water, dw is the density of water at temperature, ns is the no. of drops of solvent.

3.5 Degaradation Studies

3.5.1 Biological Oxygen Demand (BOD)

BOD the measure of the quantity of decay able organic matter present in the water. Therefore, a low BOD value is associate degree indicator of excellent quality water, whereas a high BOD value indicates impure water. Dissolved chemical element (DO) is consumed by microorganism once massive amounts of organic matter from waste product or different discharges square measure gift within the water. DO is that the actual quantity of chemical element offered in dissolved kind within the water. Once the DO drops below a precise level, the life forms in this water are unable to continue to grow at a traditional rate, this is one in all the foremost common measures of waste matter organic material in water. As a sample waste water from the laundary was collected on Day1 and its (DO) was measured in the laboratory. Six samples containg 1% of enzymes lipase with different surfactant concentrations i.e SDS, CTAB, Tween 20 and six samples containg 1% of enzymes amylase with different surfactant concentrations i.e SDS, CTAB, Tween 20 was added to twelve different glass bottles containg only waste water from the laundary. The glass bottles were then placed for incubation at 20°C for five days. On the fifth day (DO) of all twelve bottles was measured and thus results were evaluated.

Fig 3.2 Sample bottles with 12 different sample for BOD studie

CHAPTER 4

RESULTS AND DISCUSSIONS

The results obtained from thermodynamic studies, kinetic studies, surface tension studies and degradation studies have been reported in this chapter.

4.1 Conductivity studies and determination of CMC

The experimentally determined specific conductance values of various surfactants i.e SDS, CTAB and Tween 20 in the presence of enzymes amylase and lipase were measured which were then used to determine the CMC for each surfactant i.e SDS, CTAB and Tween 20 in the presence of 1% amylase and in the presence of 1% lipase. CMC values have been further used to calculate ΔH°m, ΔG°m, ΔS°m values which have been summarized for 1% amylase in **Table 4.1- 4.3** and with 1% lipase in **Table 4.4 - 4.6.** The negative value of ΔH°m , ΔG°m and positive value of ΔS°m shows the enzyme – surfactant interaction.

The influence of temperature on degree of micellization of the various surfactants was also evaluated with 1% amylase in **Table 4.1- 4.3** and with 1% lipase in **Table 4.4 - 4.6.** The value of CMC was found to fluctuate with different temperatures (25, 30, 35, 40°C) and with changing concentration of the surfactant for each surfactant (SDS, CTAB, Tween 20) in presence of different enzymes, amylase and lipase.

Table 4.1 1% Amylase with different in SDS conc. at different temperature, T=25, 30, 35, 40 ºC.

Temp °C	CMC	Xcmc	T^2	Ln Xcmc	DLn Xcmc	ΔH°m (kJ mol^{-1})	ΔG°m (kJ mol^{-1})	ΔS°m (J mol^{-1} K^{-1})
25	0.0054	9.71478E-05	625	-5.22136	0.022	-0.11432	-1.08526	0.038838
30	0.0053	9.5349E-05	900	-5.24005	0.022	-0.16462	-1.30697	0.038079
35	0.0051	9.17512E-05	1225	-5.27851	0.022	-0.22406	-1.536	0.037484
40	0.005	8.99523E-05	1600	-5.29832	0.022	-0.29265	-1.76201	0.036734

Table 4.2 1% Amylase with different C-TAB conc. at different temperature, T=25, 30, 35,40 ºC

Temp °C	CMC	Xcmc	T^2	Ln Xcmc	DLn Xcmc	ΔH°m (kJ mol^{-1})	ΔG°m (kJ mol^{-1})	ΔS°m (J mol^{-1} K^{-1})
25	0.00073	1.3134E-05	625	-7.22247	0.022	-0.11432	-1.50119	0.055475
30	0.00075	1.34939E-05	900	-7.19544	0.022	-0.16462	-1.79469	0.054336
35	0.00077	1.38537E-05	1225	-7.16912	0.022	-0.22406	-2.08614	0.053202
40	0.0008	1.43935E-05	1600	-7.1309	0.022	-0.29265	-2.37145	0.05197

Table 4.3 1% Amylase with different TWEEN 20 conc. at different temperature, T=25, 30, 35, 40 ºC

Temp °C	CMC	Xcmc	T^2	Ln Xcmc	DLn Xcmc	ΔH°m (kJ mol^{-1})	ΔG°m (kJ mol^{-1})	ΔS°m (J mol^{-1} K^{-1})
25	0.000067	1.20547E-06	625	-9.61082	0.022	-0.11432	-1.99761	0.075332
30	0.00008	1.43936E-06	900	-9.43348	0.022	-0.16462	-2.3529	0.072943
35	0.000058	1.04354E-06	1225	-9.75507	0.022	-0.22406	-2.83863	0.074702
40	0.000073	1.31342E-06	1600	-9.52505	0.022	-0.29265	-3.16765	0.071875

Table 4.4 1% lipase with different SDS conc. at different temperature, T=25, 30, 35, 40 ºC

Temp °C	CMC	Xcmc	T^2	Ln Xcmc	DLn Xcmc	ΔH°m (kJ mol^{-1})	ΔG°m (kJ mol^{-1})	ΔS°m (J mol^{-1} K^{-1})
25	0.0084	0.000151111	625	-4.77952	0.022	-0.11432	-0.99342	0.035164
30	0.0082	0.000147513	900	-4.80362	0.022	-0.16462	-1.19812	0.03445
35	0.0082	0.000147513	1225	-4.80362	0.022	-0.22406	-1.39781	0.033536
40	0.0085	0.000152909	1600	-4.76769	0.022	-0.29265	-1.58554	0.032322

Table 4.5 1 % lipase with different C-TAB conc. at different temperature, T=25, 30, 35, 40 ºC

Temp °C	CMC	Xcmc	T^2	Ln Xcmc	DLn Xcmc	ΔH°m (kJ mol^{-1})	ΔG°m (kJ mol^{-1})	ΔS°m (J mol^{-1} K^{-1})
25	0.00093	1.67324E-05	625	-6.98033	0.022	-0.11432	-1.45086	0.053462
30	0.00093	1.67324E-05	900	-6.98033	0.022	-0.16462	-1.74103	0.052547
35	0.00092	1.65524E-05	1225	-6.99114	0.022	-0.22406	-2.03435	0.051723
40	0.00097	1.7452E-05	1600	-6.93821	0.022	-0.29265	-2.30737	0.050368

Table 4.6 1% lipase with different TWEEN 20 conc. at different temperature,T=25, 30, 35, 40 ºC

Temp °C	CMC	Xcmc	T^2	Ln Xcmc	DLn Xcmc	ΔH°m (kJ mol^{-1})	ΔG°m (kJ mol^{-1})	ΔS°m (J mol^{-1} K^{-1})
25	0.000065	1.16948E-06	625	-9.64112	0.022	-0.11432	-2.00391	0.075584
30	0.000063	1.1335E-06	900	-9.67238	0.022	-0.16462	-2.41248	0.074929
35	0.000065	1.16948E-06	1225	-9.64112	0.022	-0.22406	-2.80547	0.073755
40	0.000061	1.09752E-06	1600	-9.70464	0.022	-0.29265	-3.22737	0.073368

3.2 Thermodynamic studies

The thermodynamic parameters ΔH°m, ΔG°m, and ΔS°m were evaluated using CMC values and have been summarized for 1% amylase in **Table 4.1- 4.3** and with 1% lipase in **Table 4.4 - 4.6. Fig. 4.1** shows the post micellar region, pre micellar region and CMC which is evaluated using specific conductance.

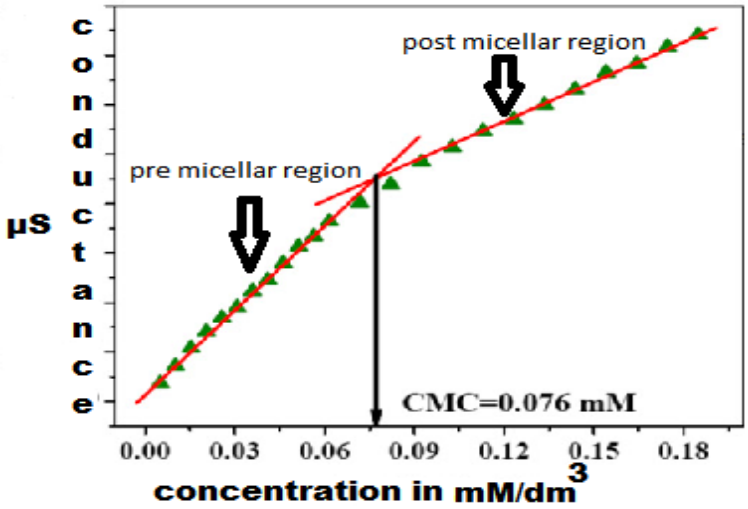

Fig. 4.1 Plot showing the post micellar region, pre micellar region and CMC.

The experimentally determined CMC values of SDS in the presence of 1% amylase shown in the **Fig. 4.2** were found to lie in the range of (5.0 - 5.4) mM. **Fig. 4.3** CMC values of CTAB in the presence of 1% lipase were found to lie in the range of (0.92 - 0.97) mM. The influence of temperature on degree of micellization of SDS was also evaluated in **Table 4.1** The value of CMC was found maximum at a temperature of 25ºC for SDS with amylase and 40ºC for CTAB with lipase. With increase in temperature the value of CMC was found to decrease. Specific conductance was found to increase with increasing concentration of SDS and of CTAB.

From the **Fig. 4.4 -4.6** the variation of ΔH°m (the standard Enthalpy for micellization), ΔG°m is the standard Gibbs free energy change of micellization, ΔS°m is the standard Entropy of micellization. at different temperatures T=25, 30, 35, 40 ºC with different surfactants (SDS, CTAB, TWEEN 20) can be seen wherein the ΔH°m value, ΔG°m value and ΔS°m value tend to decrease with increase in temperature for the different surfactants SDS,CTAB AND TWEEN 20.

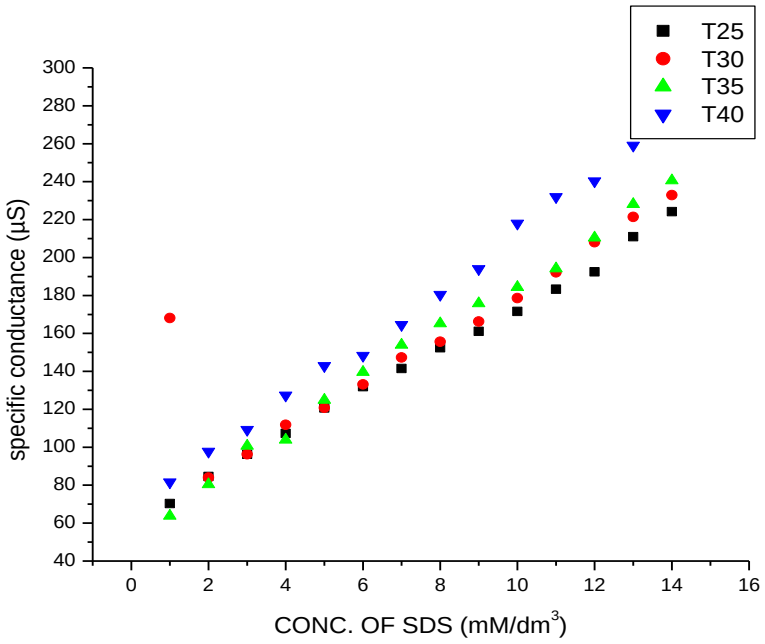

Fig.4.2 The plot of specific conductance versus SDS concentration in 1% solution of Amylase at temperatures T=25, 30, 35, 40 ºC.

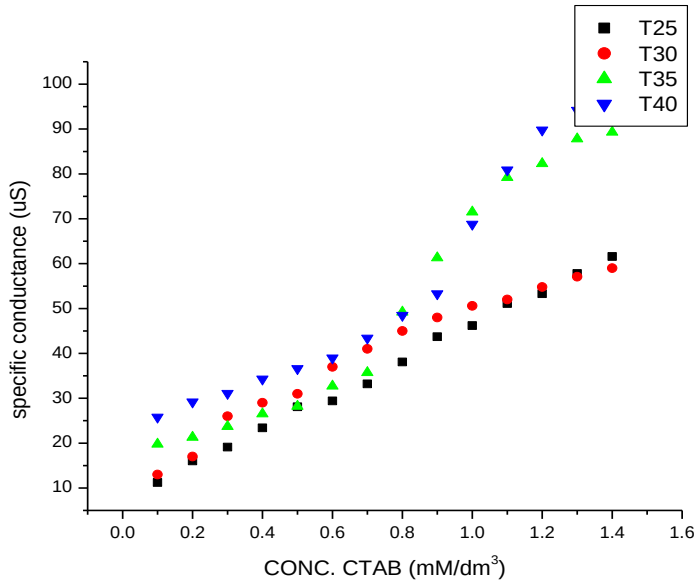

Fig.4.3 The plot of specific conductance versus CTAB concentration in 1% solution of lipase at temperatures T=25, 30, 35, 40 ºC.

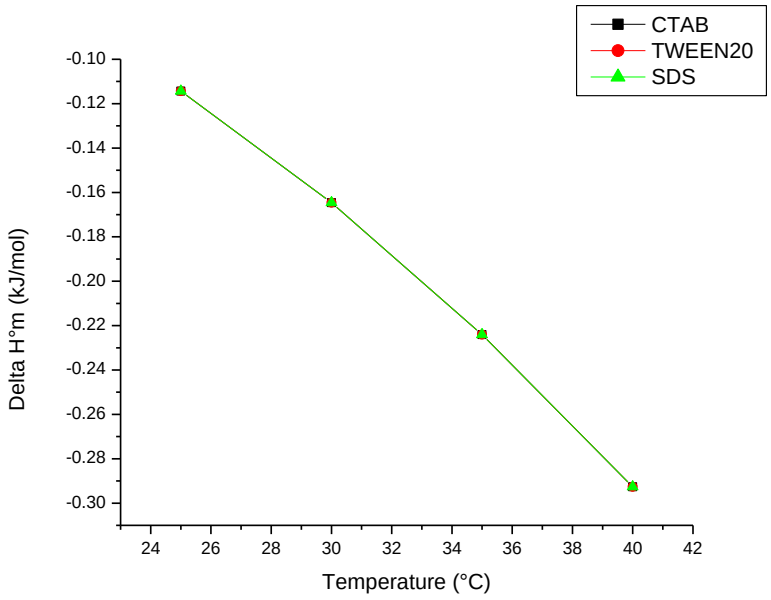

Fig.4.4 The plot of ΔH°m (the standard Enthalpy for micellization) at different temperatures T=25, 30, 35, 40 ºC with different surfactants (SDS, CTAB, TWEEN 20.

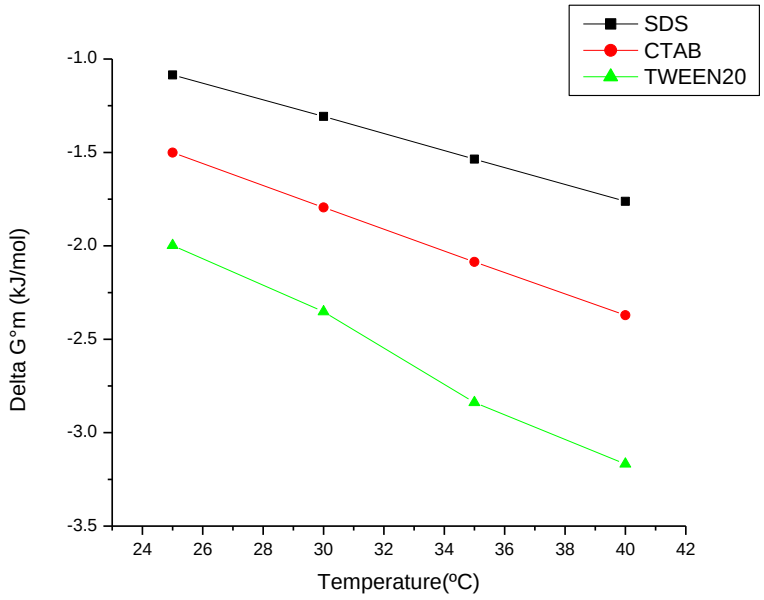

Fig.4.5 The plot of ΔG°m (the standard Gibbs free energy change of micellization) at different temperatures T=25, 30, 35, 40 ºC with different surfactants (SDS, CTAB, TWEEN 20)

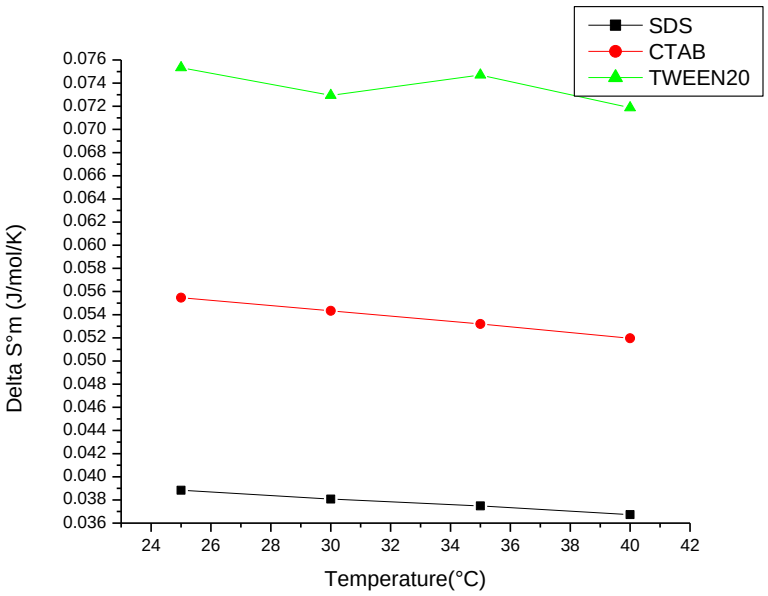

Fig. 4.6 The plot of ΔS°m (the standard Entropy of micellization) at different temperatures T=25, 30, 35, 40 ºC with different surfactants (SDS, CTAB, TWEEN 20.

4.2 KINETIC STUDIES

The Michaelis-Menten kinetic was used to study the kinetic parameters. Enzyme activity was calculated by the DNS method which is summarized in **Table 4.2.1** the recorded absorbance at 540nm was used to obtain a standard plot shown in **Fig. 4.2.1.** The equation thus obtained from the standard plot was further used to calculate the enzyme activity at different conc. of surfactants SDS, CTAB and Tween 20.

Table 4.2.1 preparation of reaction to obtain standard plot to obtain enzyme activity.

S. NO	Glucose conc.(mg/ml)	Glucose Vol. (µl)	Distilled water (µl)	Total vol. (ml)	Incubation at 37 °C for 20 min.	DNS (ml)	Incubation at 100° C for 10 min.	Distilled water (ml)	Absorbance in 96 well plate at 540
1	0	0	1000	1		3		5	
2	0.1	100	900	1		3		5	
3	0.2	200	800	1		3		5	
4	0.4	400	600	1		3		5	
5	0.6	600	400	1		3		5	
6	0.8	800	200	1		3		5	
7	1	1000	0	1		3		5	

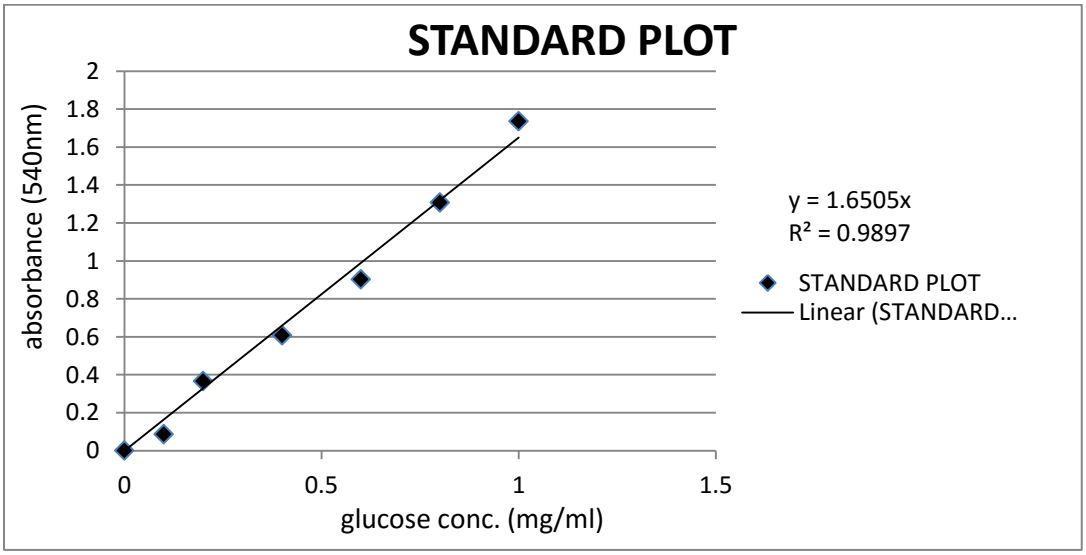

Fig. 4.2.1 The standard plot of glucose concentration versus absorbance at 540 nm used as a standard to obtain the activity of an enzyme.

Enzyme activity:

The equation obtained as y =1.650 x is used to calculate the value of x (amt. of glucose released) (y denotes absorbance). The following equation is used thereafter to calculate enzyme activity.

$$\text{Enzyme activity} = \frac{\text{amount of glucose released (mg/ml)} \times 1000}{\text{Volume of enzyme used} \times \text{incubation time} \times \text{mol. Wt of Glucose}}$$

From the **Fig. 4.2.2 – 4.2.4** activity of enzyme have been determined for each surfactants. The concentration values for each surfactant determined from kinetic studies (where activity is maximum) was found to be similar to the value of CMC determined from thermodynamic studies. Series of experiments done and results show that the study can be used for potential detergent formulation.

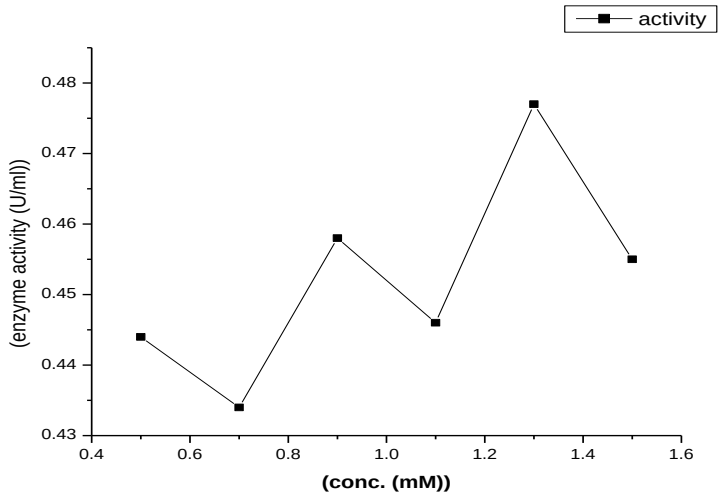

Fig. 4.2.2 Plot of concentration of CTAB versus enzyme activity representing various conc. values.

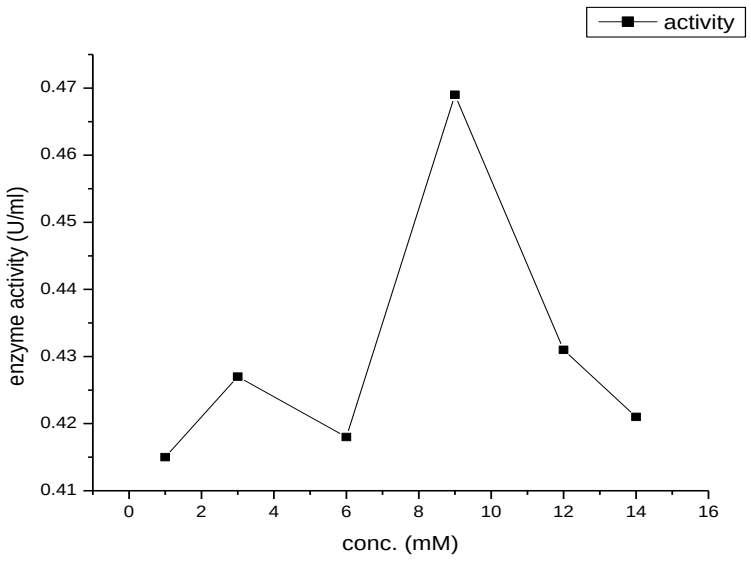

Fig. 4.2.3 Plot of concentration of SDS versus enzyme activity representing various conc. values.

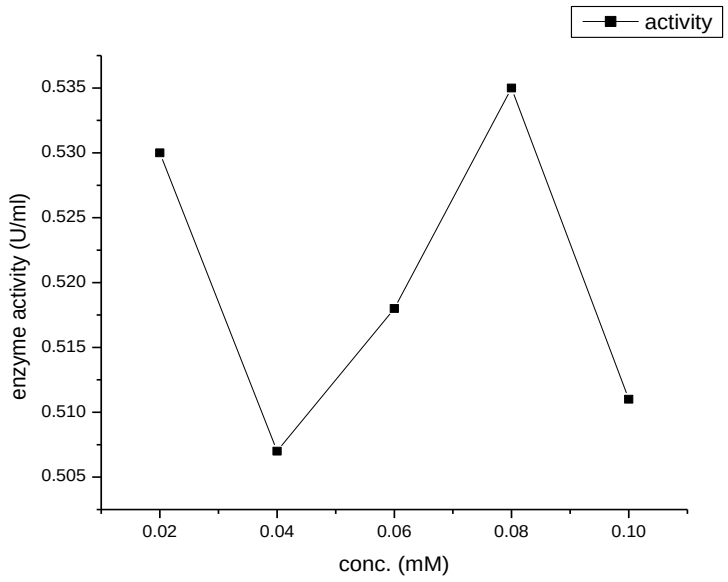

Fig. 4.2.4 Plot of concentration of TWEEN 20 versus enzyme activity representing various conc. values.

4.3 Surface tension

The development is employed to see the physical phenomenon exploitation stalagmometric methodology (method of reckoning drops). . To determine the surface tension of the sample i.e a liquid, fill the stalagmometer with the sample upto to upper marked point by sucking the sample into the stalgmometer through the rubber tube attached to the upper part of the stalgmometer and then permit the liquid to flow freely by slowly releasing the screw on the rubber tube attached to the stalgmometer to control the flow of the liquid. While the sample flows out of the stalgmometer count the number of drops for each sample. Now repeat the procedure with the samples at different temperatures (25, 30, 35, 40°C) and record the number of drops for each sample with varying temperatures.

The no. of drops are recorded for each sample of amylase and lipase with SDS, CTAB and Tween 20 at different tempeartures of 25, 30, 35 and 40°C. The density for each sample at a specific conc. and temperature is calculated in gm/cm^3 which was further used to calculate the surface tension of the sample at that conc. theoretical values of density of water and viscosity of water at a particular temperature was also used in the above calculations.

From the **Fig.(4.3.1 – 4.3.6)** the variation of each surfactant SDS, CTAB, Tween20 at different conc. (3,6,9,12 and 15 mM/dm^3) is seen. The **fig. (4.3.1 – 4.3.6)** also shows the variation of each surfactant SDS, CTAB, Tween20 at different conc. with different temperatures (25,30,35 and 40°c).in **fig (4.3.1 – 4.3.3)** variation is seen in the presence of 1% solution of enzyme amylase and in **fig (4.3.4 – 4.3.6)** variation is seen in the presence of 1% solution of enzyme Lipase. The conc. at which the surface tension value is least **fig. (4.3.1 – 4.3.6).** That conc. was found to be similar to the CMC values calculated in the CMC determination studies.

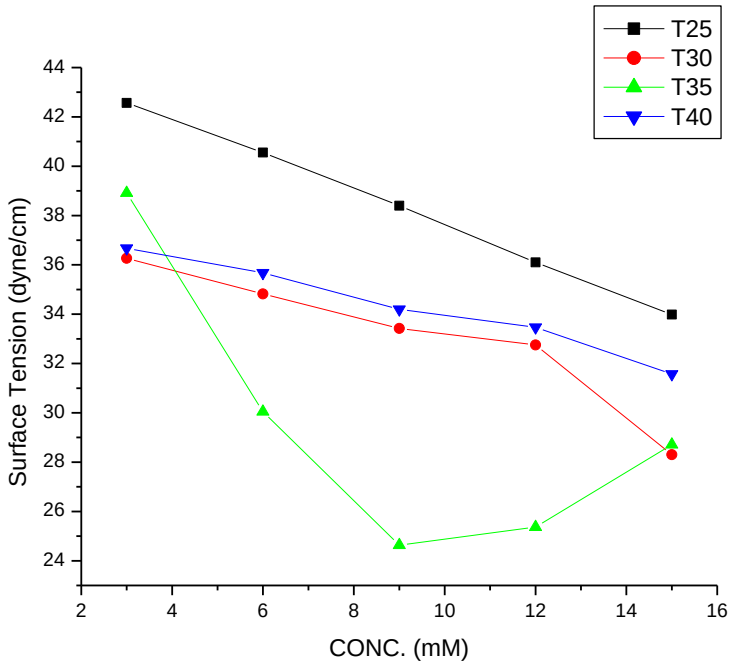

Fig. 4.3.1 The plot of surface tension versus SDS concentration in 1% solution of Amylase at temperatures T=25, 30, 35, 40 °C.

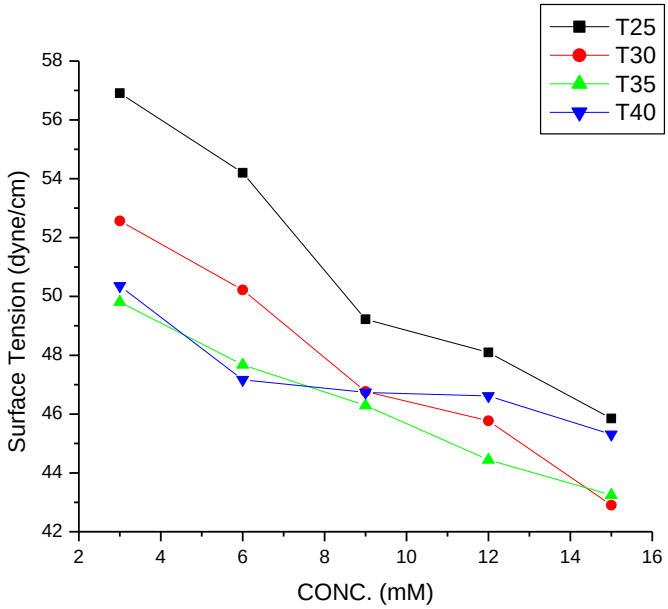

Fig. 4.3.2 The plot of surface tension versus CTAB concentration in 1% solution of Amylase at temperatures T=25, 30, 35, 40 °C.

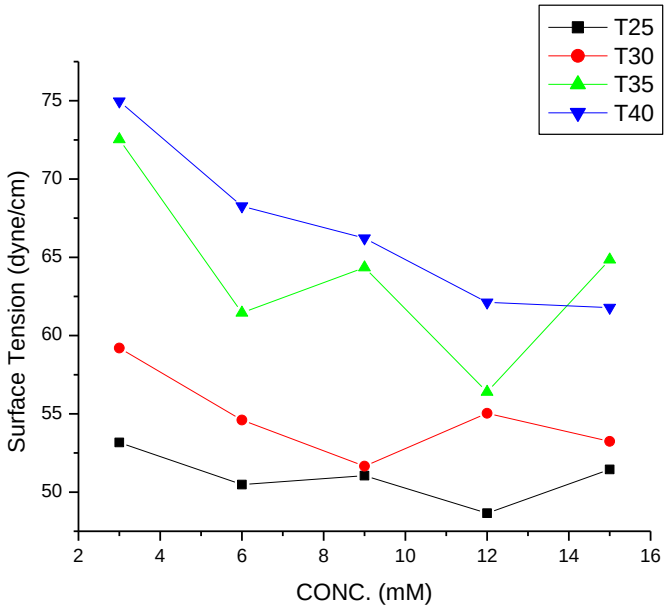

Fig. 4.3.3 The plot of surface tension versus TWEEN 20 concentration in 1% solution of Amylase at temperatures T=25, 30, 35, 40 ºC.

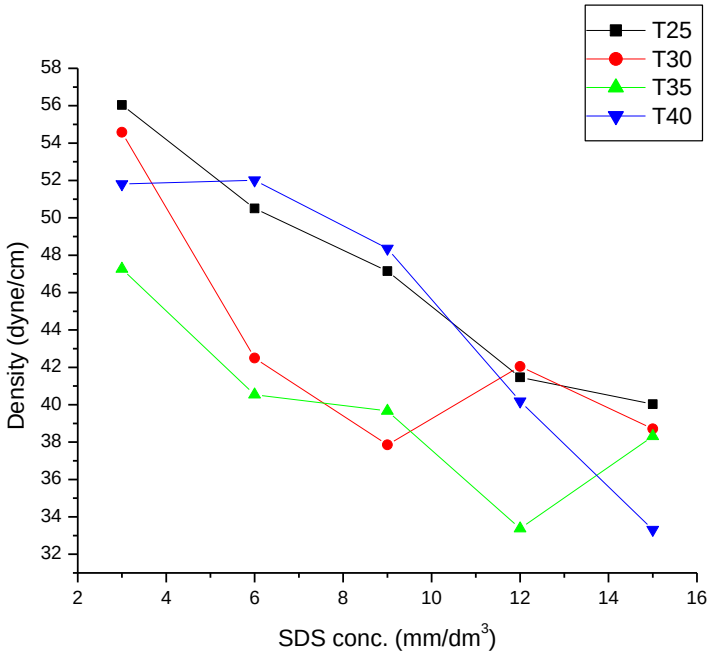

Fig. 4.3.4 The plot of surface tension versus SDS concentration in 1% solution of Lipase at temperatures T=25, 30, 35, 40 ºC.

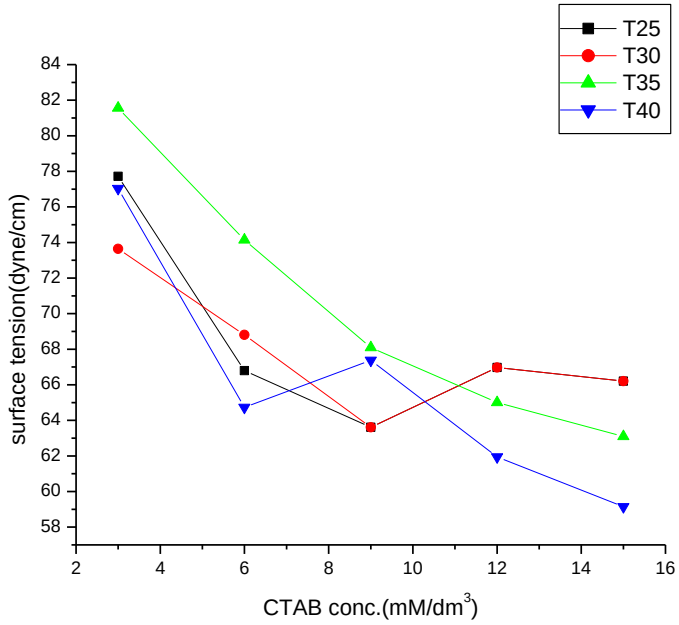

Fig. 4.3.5 The plot of surface tension versus CTAB concentration in 1% solution of Lipase at temperatures T=25, 30, 35, 40 ºC.

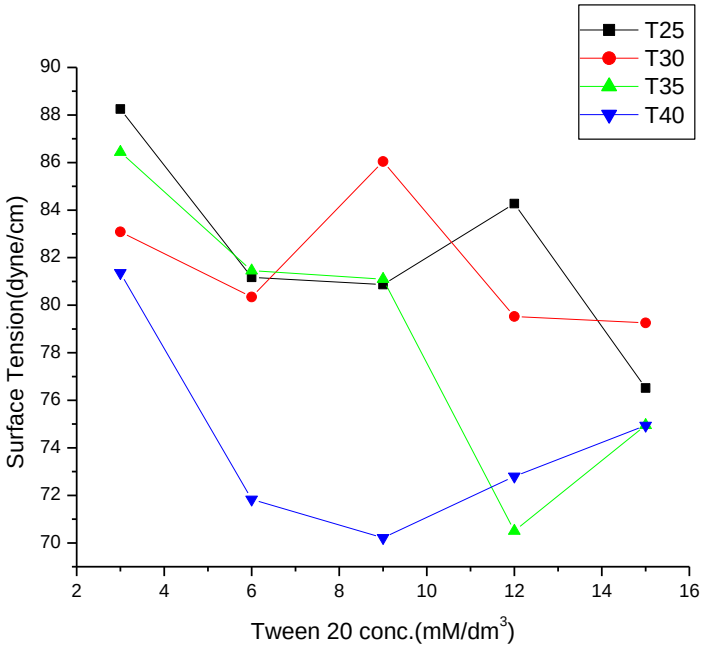

Fig. 4.3.6 The plot of surface tension versus TWEEN20 concentration in 1% solution of Lipase at temperatures T=25, 30, 35, 40 ºC.

4.4 Degradation studies

Measure of the dissolved oxygen (DO) was taken from the sample from the laundary, collected on Day1. Six samples containg 1% of enzymes lipase with different surfactant concentrations i.e SDS, CTAB, Tween 20 and six samples containg 1% of enzymes amylase with different surfactant concentrations i.e SDS, CTAB, Tween 20 was added to twelve different glass bottles containg only waste water from the laundary.

The surfactant conc. that was to be added to the sample was obtained from the previous study such that the conc. is similar to the CMC value of the surfactant. The glass bottles were then placed for incubation at 20°C for five days. On the fifth day (DO) of all twelve bottles was measured and biological oxygen demand (BOD) for each sample was calculated. Sample no 11 and 7 had the minimum BOD, therefore this conc. can be used for detergent formulation.

Table 4.4 Calculation of biological oxygen demand for 12 samples containing different conc. of enzymes (lipase and amylase) and surfactants (SDS, CTAB, Tween 20)

SAMPLE BOTTLE No.	**D.O on Day 1 (mg/L)**	**D.O on Day 5 (mg/L)**	**B.O.D** $= \frac{(\text{D.O initial} - \text{D.O final})}{Dilution\ Factor}$
1	7.02	1.64	46.38
2	7.02	1.70	51.03
3	7.02	1.65	46.29
4	7.02	1.95	43.71
5	7.02	1.67	46.12
6	7.02	1.99	43.36
7	7.02	2.19	41.64
8	7.02	1.61	46.64
9	7.02	1.27	49.57
10	7.02	1.86	44.48
11	7.02	3.05	34.22
12	7.02	0.74	54.14

CHAPTER 5

CONCLUSION

The study conducted for the formulation of "Green Detergent" by physico-chemical properties, conductivity study, thermodynamic study initially to locate the region of micellization between the surfactants i.e anionic surfactant sodium dodecyl sulphate (SDS), cationic surfactant cetyl trimethylammonium bromide (CTAB) and non-ionic surfactant (TWEEN 20) in presence of enzyme α-amylase, from *Aspergillus oryzae* and enzyme lipase from *Aspergillus niger* at completely different temperatures 25, 30, 35, 40ºC. Further supporting studies have been performed i.e surface tension study and enzyme activity with the help of Michaelis-Menten mechanics. Further degradation studies have been performed to check the biodegradability of conc. of surfactants and enzymes for which BOD was found to be minimum for amylase and tween and amylase and SDS. Thus this conc. of the amylase and surfactants can be further used for detergent formulation thus increasing the degradability without compromising with the cleaning efficiency of the detergent.

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PAPER PRESENTATION

Paper presentation in national conference *"Innovations & Challenges in Basic & Applied Sciences – (ICBAS-2017)"* at Maharaja Agrasen University, baddi.

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