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The Effect Of Lipase Enzyme on Linear Alkylbenzene Sulfonate (LAS) and Nonyl Phenol Ethoxylates (NP6) in Laundry Detergents

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Thesis Approval

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Jerusalem – Palestine

Dedication

I wish to dedicate this thesis to my family, wife and children and all my friends specially Hakam Khanfar, Ahmad Kassarweh and Khalid Zeyada.

Lu'ai Mohammed Ahmad Darwiesh

Declaration

I certify that this thesis submitted for the degree of master, is the result of my own research, except where otherwise acknowledged, and this thesis has not been submitted for the higher degree to any others university or institution.

Signed : …….

Lu'ai Mohammed Ahmad Darwiesh.

Date : 17 / 6 / 2009.

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Abstract

Detergency is one of the most important modern industries because of its importance in our daily life. The most important detergent is washing powders. It contains different sequences of surfactants , enzymes and others. Such materials like LAS ionic and NP6 nonionic surfactants and the Lipolase enzyme. These are the subject of this search.

The question in this Thesis is how hard dirt can be removed especially the fat, from clothes by using as low concentrations as possible of surfactants and enzymes? Reducing the temperature of the water used for cleaning? Because of the high prices of these materials and the cost of production. And the selection of optimal values for these variables that help in the process of washing.

Olive oil was chosen as a source of fatty dirt. Lipolase 100T enzyme was used for the removal of the dirt from cloth structures by washing powders containing LAS and NP6. Changing the conditions of temperature and PH values of solutions of these materials. we studied the effects of these materials through preparation of solutions with different concentrated. This installation hydrolysed of olive oil to fatty acids. It in turn reduced the value of PH, and then the percentages of fatty acids in the solutions to which it belongs were determined.

The effectiveness of the enzyme, the selection of optimal conditions and the combinations of solvents for cleaning process were calculated, well as the impact of the type of surfactant on the effectiveness of enzyme was identified.

On the other hand, many pieces of different cloth are washed with the best different solvents by using a model of washing machine that has been manufactured specifically for this study to determine the effectiveness of enzyme in the process of washing by observing the differences between the washed and dirty pieces of cloth.

The study showed that the enzyme Lipolase 100T has a positive impact on the effectiveness of detergents. It has high effectiveness by itself and high concentration without any kind of

surfactant, while it is less effective in the presence of a high concentration of LAS, as well as It is less effective in the presence of NP6 but less than that of LAS, which is unusual for non-ionic surfactant but it often increases the effectiveness of enzymes.

Temperature studies were attempted in order to discern the optimum temperature needed for efficiency of enzyme.

Temperature between $50^{o}C$ – $60^{o}C$, and under normal medium (PH7) was the best effectiveness of the enzyme. The effectiveness was low with increasing values of PH. The enzyme stability by itself is obvious without surfactant in a wide range of pH (7 - 10).

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* Figure 3.9 has been excluded from the list to apply Table No. to the No. diagram accompanying

At Lipolase (100T) 0.05 concentration

At Lipolase (100T) 0.01 concentration

Definitions

%	Percentage
Conc.	Concentrations
Fig.	Figure
g	Gram
LAS	Linear alkylbenzene sulfonate
mL	Milliliter
No.	Number
NP6	Nonyl phenol ethoxylates 6
^{o}C	Celsius
PH	Hydrogen Number
Surfc.	Surfactant
Sol'n	Solution
Temp.	Temperature

Part one

Introduction

1. Introduction

1.1 Enzymes

Enzymes are proteins that act as a catalysts in biological systems, composed of hundreds of amino-acids, which are produced by living organisms. They are very specific in their functions and responsible for a number of reactions and biological activities in plants, animals, human beings and micro-organisms. They are found in the human digestive system to break down carbohydrates (sugars), fats or proteins present in food, and now in detergents to wash different substrates.

1.1.1. List of Enzymes and where they are produced:

1. **Amylase**: Found in starches into maltose (a disaccharide), produced in saliva and pancreas, and released into the small intestine.
2. **Lipase**: Found in fats (lipids), produced in stomach and pancreas , and released into the small intestine.
3. **Pepsin**: Found in proteins into absorbable peptides and peptones, produced in stomach.
4. **Gelatinase**: Found in gelatin, produced in stomach.
5. **Maltase**: Found in maltose into monosaccharides, produced by the pancreas, released into the small intestine.
6. **Lactase**: Found in lactose into monosaccharides, produced in small intestine.
7. **Trypson**: Found in proteins into peptides and amino acids, produced in pancreas and released into small intestine.
8. **Chymotrypson**: Found in proteins into peptides and amino acids, produced in pancreases and released into small intestine.

Each enzyme is made of a sequence of amino acids folded into a unique three-dimensional structure that determines the function of the enzyme. Even the slightest change in the sequence of the amino acids can alter the shape and function of the enzyme.

1.1.2. Production of Enzymes:

Enzyme molecules are complex to synthesize by purely chemical means, and so the only way to make them is to use living organisms mean by gene manipulation technology, but There is a problem, that enzymes produced by micro-organisms in the wild are often expressed in tiny amounts and mixed up with many other enzymes and proteins. These micro-organisms can also be very difficult to cultivate under industrial conditions, and they may create undesirable by-products.

Enzymes are produced in modern industrial cultivation by fermentation of a vial of dried or frozen micro-organisms called a production strain. This production strain is selected to produce large amounts of the enzymes of interest. See Figure 1.1:

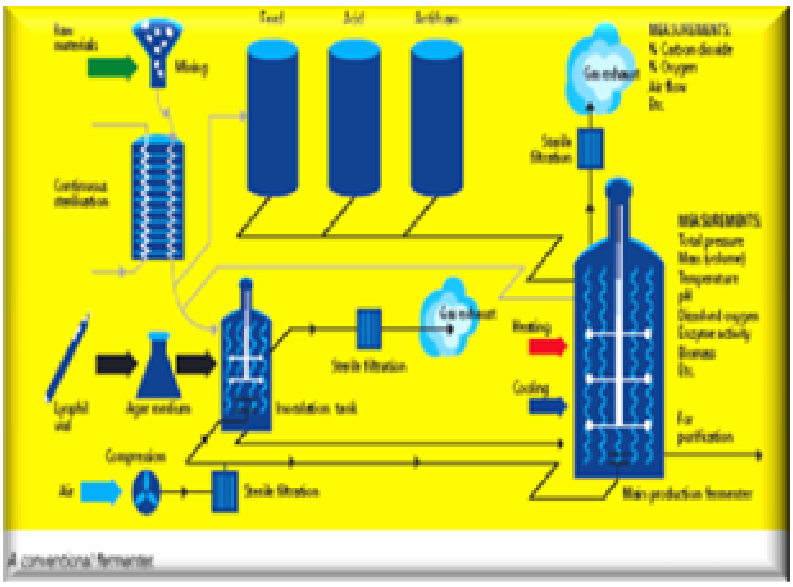


Figure 1.1 : A conventional fermented

1.2 Surfactants

A surfactant - a shortened form of "surface-active agent" – is a chemical that stabilizes mixtures of oil and water by reducing the surface tension at the interface between the oil and water molecules and that because water and oil do not dissolve in each other . Surfactant has

to be added to the mixture to keep it from separating into layers. Surfactants can be used in different fields like cosmetics and detergents to provide one or more of six functions: (dermatology.about2007).

1. Detergents - for cleansing.
2. Wetting agents - in perms.
3. Foaming agents - for shampoos.
4. Emulsifiers - in creams and lotions.
5. Conditioning agents - in skin and hair-care products.
6. Solubilizers - for perfumes and flavors.

1.2.1. Classification of surfactants:

From the commercial point of view surfactants are often classified according to their use. However, this is not very useful because many surfactants have several uses, and confusions may arise from that. The most accepted and scientifically sound classification of surfactants is based on their dissociation in water, and these types are (Jean-Louis Salager 2008):

1.2.1.1. Anionic surfactants:

Anionic Surfactants are dissociated in water in an amphiphilic anion, and a cation, which is in general an alkaline metal (Na^+, K^+) or a quaternary ammonium. They are the most commonly used surfactants. They include alkylbenzene sulfonates (detergents), (fatty acid) soaps, lauryl sulfate (foaming agent), di-alkyl sulfosuccinate (wetting agent), lignosulfonates (dispersants) etc… Anionic surfactants account for about 50 % of the world production (Jean-Louis Salager 2008).

1.2.1.2. Nonionic Surfactants:

Nonionic Surfactants come as a close second with about 45% of the overall industrial production. They do not ionize in aqueous solution, because their hydrophilic group is of a nondissociable type, such as alcohol, phenol, ether, ester, or amide. A large proportion of these nonionic surfactants are made hydrophilic by the presence of a polyethylene glycol chain,

obtained by the polycondensation of ethylene oxide. They are called polyethoxylated nonionic's**.**

In the past decade glucoside (sugar based) head groups, have been introduced in the market, because of their low toxicity. As far as the lipophilic group is concerned, it is often of the alkyl or alkylbenzen type, the former coming from fatty acids of natural origin. The polycondensation of propylene oxide produce a polyether which (in opposition to polyethylene oxide) is slightly hydrophobic (Jean-Louis Salager 2008).

Block copolymers, which are most of ten included in a different class, e.g. polymeric surfactants, to be dealt with later.

1.2.1.3. Cationic surfactants:

Cationic Surfactants are dissociated in water into an amphiphilic cation and an anion, most often of the halogen type. A very large proportion of this class corresponds to nitrogen compounds such as fatty amine salts and quaternary ammoniums, with one or several long chain of the alkyl type, often coming from natural fatty acids. These surfactants are in general more expensive than an ionic's, because of the high pressure hydrogenation reaction to be carried out during their synthesis. As a consequence, they are only used in two cases in which there is no cheaper substitute, one as bactericide, two as positively charged substance which is able to adsorb on negatively charged substrates to produce antistatic and hydrophobant effect, often of great commercial importance such as in corrosion inhibition (Jean-Louis Salager 2008).

1.2.1.4. Amphoteric or **zwitterionic surfactants:**

When a single surfactant molecule exhibit both anionic and cationic dissociations it is called ampHoteric or zwitterionic. This is the case of synthetic products like betaines or sulfobetaines and natural substances such as amino acids and phospholipids (Jean-Louis Salager 2008).

1.3 Detergents

The term “detergents” is applied to materials or products that provide the following functions: (Michael S. Showell 2006).

1. Promote removal of material from a surface like food from a dish, soap scum from a hard surface or soil from a fabric.
2. Disperse and stabilize materials in a bulk matrix like suspension of oil droplets in a mobile phase like water.

A detergent (as a noun) is a material intended to assist cleaning. The term is sometimes used to differentiate between soap and other surfactants used for cleaning. As an adjective pertaining to a substance, it means cleaning or having cleaning properties. Detergency indicates presence or degree of cleaning property (Ochoa-Gomez 1996)**.**

Detergents are designed for specific purposes. A laundry detergent has a different makeup from a floor or hard surface detergent. Some detergents come in Liquid form and others are sold as powders.

The basic ingredient in detergent, called a surface-active agent (surfactant), is synthetic, meaning that it is chemically constructed from raw materials other than soap. This eliminates the problems associated with soaps while retaining the needed wetting, emulsifying, and suspension capabilities. A builder is usually added to inactivate water hardness.

1.3.1. The most common and familiar detergents:

The most common and familiar detergents are those used in household cleaning and personal care. These products can be grouped into four general categories (Michael Showell 2006).

1. **Laundry detergents and laundry aids**: These comprise mainframe laundry detergents in powder, liquid, Tablet, gel, and bar form, fabric conditioner products typically in liquid

or sheet form, and an array of specialty products like sticks, sprays, bars, presoaks (liquids, powders), and bleaches (liquids, powders) (Michael. Showell 2006).

Typical laundry detergents are formulated to provide general cleaning, which includes removal of soils and stains as well as the ability to giving whiteness and brightness. In addition, many premium laundry detergents offer additional benefits like fabric softening, dye lock, and fiber protection (Michael Showell 2006).

2. **Dishwashing products**: These include detergents for hand and machine dishwashing and are typically provided in liquid, gel, powder, or Tablet form. Hand dish wash products are formulated to remove and suspend food soils from a variety of surfaces. They also must deliver long-lasting suds, even at high soil loads, and they must be mild to skin.

   Products designed for automatic dishwashing must provide soil removal and suspension, control of water hardness and sheeting of water off dish surfaces in order to achieve a spot - and film - free finish, and produce little or no suds that would otherwise interfere with the operation of the machine.

   Rinse aids are specialty detergent formulations for automatic dishwashing designed to promote drainage of water from surfaces via lowering of surface tension. This helps minimize spotting and filming during drying (Michael Showell 2006).

3. **Household cleaning products**: These are typically formulated either in liquid or powder form although gel, solid, sheet, and pad products are also available. So called “all-purpose” cleaners are designed to penetrate and loosen soil, control water hardness, and prevent soil from re depositing onto clean surfaces. Many of these products also contain low levels of antibacterial actives like triclosan.

   Powdered abrasive cleaners remove heavy accumulations of soil via the use of mineral or metallic abrasive particles. Some of these products may also bleach and disinfect

through the incorporation of a bleach precursor like sodium perborate, sodium percabonate, or sodium dichloroisocyanurate (Michael . Showell 2006).

4. **Personal cleansing products**: These include products for hand and body washing as well as shampoos, conditioners, and toothpastes. They are marketed primarily in bar, gel, and liquid forms. A major consideration in formulation of such products is the desired consumer aesthetic such as lather, skin feel, smell, and taste.

Formulations designed for cleaning may also provide moisturizing benefits, conditioning, and styling effects (Michael. Showell 2006).

1.3.2. Other applications and industries for detergents:(Michael.Showell 2006)

1. Environmental remediation : Surfactant systems have been developed to aid in the clean up of contaminated groundwater supplies.

2. Enhanced oil recovery: Micellar and surfactant are among the most successful methods of enhancing recovery of oil from depleted reservoirs.

3. Nanoegineering: Researchers have used the phase behavior of surfactants to generate self-assembling nanosystems.

4. Formulation of paints and printing inks : Paints and inks comprise formulations wherein a pigment is dispersed into a liquid phase. The dispersion is typically achieved with surfactants and/or dispersing polymers .

5. Preparation and application of synthetic polymers: Emulsion polymerization and the preparation of latexes represent one of the largest uses for surfactants outside the cleaning arena.

6. Industrial metal parts cleaning: Detergent compositions based on a CO_2 bulk phase have application in the cleaning of microelectronic components.

7. Medical applications: Mimics of human lung surfactants have been developed to treat respiratory distress syndrome in premature infants.

8. Lubricants: While highly diverse, lubricant formulations utilize the same basic additives: surfactants, dispersants, anti wear actives, antioxidants, corrosion inhibitors, and viscosity modifiers.

9. Textile processing: Detergent formulations are used to clean fibers prior to manufacture into finished textiles as well as lubricate the fibers during spinning and weaving.

10. Agricultural preparations: Pesticide and herbicide preparations are often formulated as aqueous dispersions with specific functional actives to promote even distribution of the active during application and fast penetration of the active upon contact with plants .

1.3.3. Composition of detergents:

Modern detergents can comprise 20 or more ingredients depending on what benefits the detergent is meant to deliver

Detergents, especially those made for use with water, often include different components such as (Michael . Showell 2006)**:**

- Surfactants are the most common ingredient of the detergent formulations, to 'cut' (Emulsify) grease and to wet surfaces.
- Abrasive to scour.
- Substances to modify PH or to affect performance or stability of other ingredients, acids for decaling or caustics to break down organic compounds.
- Water softeners to counteract the effect of hardness ions on other ingredients.
- Oxidants (oxidizers) for bleaching, disinfection, and breaking down organic compounds.

- Non-surfactant materials that keep dirt in suspension.
- Enzymes to digest proteins, fats, or carbohydrates in stains or to modify fabric feel.
- Ingredients that modify the foaming properties of the cleaning surfactants, to either stabilize or counteract foam.
- Ingredients to increase or decrease the viscosity of the solution, or to keep other ingredients in solution, in a detergent supplied as a water solution or gel.
- Ingredients that affect aesthetic properties of the item to be cleaned, or of the detergent itself before or during use, such as optical brighteners, fabric softeners, colors, perfumes, etc.
- Ingredients such as corrosion inhibitors to counteract damage to equipment with which the detergent is used.
- Ingredients to reduce harm or produce benefits to skin, when the detergent is used by bare hand on inanimate objects or used to clean skin.
- Preservatives to prevent spoilage of other ingredients.

Sometimes materials more complicated than mere mixtures of compounds are said to be detergent. For instance, certain foods such as celery are said to be detergent or detersive to teeth.

1.3.3. How detergents clean ?:

A surfactant is a substance that can greatly reduce the surface tension of water when used in low concentrations. They are soluble surface-active agents comprised of a hydrophobic portion, usually a long alkyl chain that is attached to hydrophilic functional groups. The hydrophilic end of the surfactant is strongly attracted to the water molecules, and the force of attraction between the hydrophobic and water is only slight. As a result, the surfactant molecules align themselves at the surface so that the hydrophilic end is towards the water, and the hydrophobic is squeezed away from the water**,** and this clear in Figure 1.2:

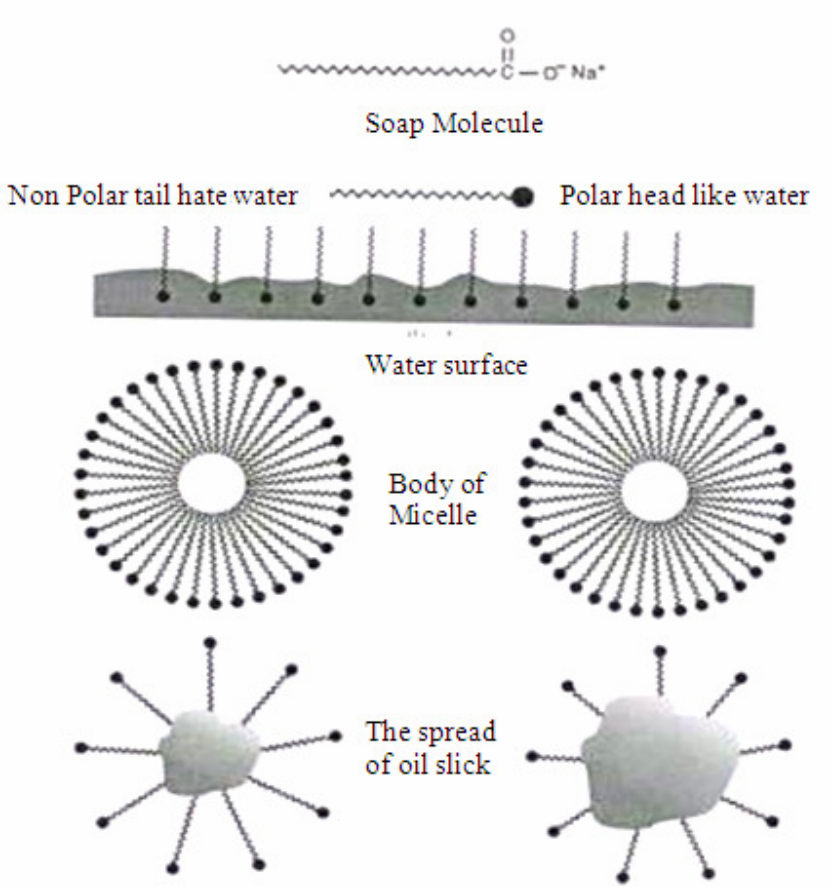


Figure 1.2: Micelle form and the manner in which the cleaning proceeds (Ghassan al-Sarhan et al. 2006).

1.3.5. Types of enzymes that can be used in cleaning products:

Four types of enzymes that can be used in cleaning and detergents, that are (Enzymes detergent 2008):

1. Protease, its the most widely and common detergents enzymes which can used to clean soils, protein stains, milk, blood …ect. (Guy Broze 1999).

   Protease catalyze and breakdown of large and complex protein molecule into peptides and amino acids which are easily removed by surfactants, see Figure 1.3.

There are many types of protease enzymes in the market and used in detergents, see Table 1.1.

Table 1.1: Main Detergents Protease Available on the market (Guy Broze 1999).

(a) Characteristics

Supplier trade name	Production type	Optimum pH	Optimum Temp. (°C)	Characteristics (according to the supplier)
Genencor				
Maxatase	Traditional	9.5–10	60	Alkaline protease
Purafect®	Gene Technology	10	60	High-pH alkaline protease
Maxacal®	Traditional	11	55–60	High-pH alkaline protease
Purafect®OxP[a]	Protein-engineered Purafect G			Oxidatively stable for bleach-containing detergents
Maxapem®	Protein-engineered Maxacal	11	45–60	Oxidatively stable for bleach-containing detergents
Properase®[b]	Protein-engineered Maxacal	11		High-pH/low-temperature wash
Novo Nordisk				
Alcalase®	Traditional	8–9	60	Very broad substrate specificity at moderate alkalinity
Savinase®	Traditional	9–11	55	Very broad substrate specificity at higher alkalinity
Durazym™	Protein-engineered Savinase®	10	45	Low cost, better stability upon aging, better stability in strong bleach system, better efficacy at low temperature
Esperase®	Traditional	9–12	55	Efficient under very alkaline conditions

2. Amylase used in detergents to remove starch based food, and used in dishwashing detergents, but to much smaller extent than protease.

   Amylase catalyze breakdown starchy molecules to hemi acetal which are easily removed by surfactants, see Figure 1.3.

   There are many types of Amylase enzymes in the market and used in detergents, see Table 1.2.

Table 1.2: Main Detergents Amylases Available on the Market (Guy Broze 1999).

(a) Characteristics

Supplier Trade name	Production type	Optimum pH	Optimum Temp. (°C)	Characteristics (according to the supplier)
Genencor				
Purafect®				Oxidatively stable, high-activity α-amylase
Maxamyl®	Genetically modified *Bacillus*	5–8.5	100	Thermostable bacterial α-amylase
Novo Nordisk				
Termamyl®		7–11	75	High stability at high temperature and alkaline pH, high activity and good stability in the presence of sequestering agents
BAN (Bacterial Amylase Novo)		7–9.5	70	Low activity and poor stability in the presence of sequestering agents and at high pH values
Duramyl 60T				

3. Lipase used in detergents to remove fatty food stains, cosmetics and oily stains, the best removed by surfactants when the soil is molten (Guy Broze 1999).

   Lipase catalyzed the hydrolysis of the ester bonds of the hydrophilic triglyceride present in oils and fats ,see Figure 1.3. (more in formation see later in section 1.5).

4. Cellulase is the last detergent enzyme type introduced on the market, it has a quite differently benefits like fabric softening, color brightening, it s used also to remove dust and mud.

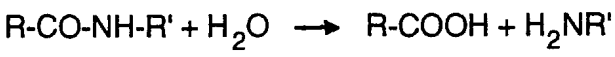
   Cellulase catalyze the hydrolysis of the β-1.4 glycosidic linkage of cellulose, see Figure 1.3.

   Cellulase have different types that found in a market. see Table 1.3.

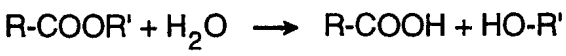
Table 1.3: Main Detergents Cellulose Available on the Market (Guy Broze 1999).

Supplier Trade name	Physical form	Available concentration	Optimum		Recommended dosage[a]
			pH	Temp. (°C)	
Novo Nordisk Celluzyme™ 0.7T	Granules	700 CEVU/g	7–9.5	50	1–3%

Proteases catalyze hydrolysis of peptide (amide) bonds



Lipases catalyze hydrolysis of ester (triglycerides) bonds



Amylases and cellulases catalyze hydrolysis of acetals to hemiacetals

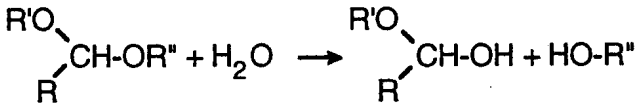


Figure 1.3: Hydrolysis reaction catalyzed by detergents enzyme (Guy Broze 1999).

Enzymes provide the superior cleaning performance needed to attack stains. Generally, the first and earliest enzymes that added to laundry detergents were proteases. However, in today's dynamic marketplace, the most successful detergent brands sometimes combine proteases with amylases, lipases and cellulases to deliver outstanding cleaning performance (Encarnacio´n Jurado, 2007).

Each of these enzymes is able to attack a specific type of stain or soil. Accordingly, the inclusion of multiple enzymes in a detergent allows the product to tackle a much broader profile of soil types. What's more, multiple enzymes can work in concert to remove tough stains or soils made up of a variety of substances.

1.3.6. Characteristics of detergents Enzymes:

Natural Enzymes that found in human body, animals and fruits not really suitable for detergents because they work in acidic or natural condition, so detergents enzymes are produced by bacterial strains which are expensive. (Guy Broze 1999)

Enzymes are sensitive to some detergents ingredients like alkylbenzene sulfonates surfactant that degraded enzymes and oxidizing agent that deactivated enzyme. (Guy Broze 1999)

Enzymes must exhibit the following properties to be suited for use in detergents: (Guy Broze 1999).

- Alkaline pH optimum.
- Efficacy at low wash temperature of 20°C-40°C.
- Stability at wash temperature up to 60°C.
- Stability in the presence of other detergents ingredients.
- Specificity broad enough to enable the degradation of a whole class of molecules.

1.4 Laundry detergents:

Laundry detergent, or washing powder, is a substance which is a type of detergent(cleaning agent) that is added when one is washing laundry to aid in getting the laundry cleaner.

Laundry detergent has traditionally been a powdered or granular solid, but the use of liquid laundry detergents has gradually increased over the years, and the popularity of liquid detergent now rivals that of solid detergent. Some brands also manufacture laundry soap in Tablets and dissolvable packets, so as to eliminate the need to measure soap for each load of laundry (Jean-Louis Salager 2008).

In some countries where washing clothes by hand is more popular, detergent bars are more popular. Recently, environmentally friendly detergents have experienced a surge in popularity (Jean-Louis Salager 2008).

1.4.1. Manufacturing and use laundry detergents:

Figure 1.4: Environmental life cycle of laundry detergents (Pentti Ja¨rvi, Ari Paloviita2007)

1.4.2. Enzymes in laundry detergents:

The use of trypsin for the first time in washing was reported in 1913 by Otto Roehm and in the course of time the use of surfactant proteases and the introduction of amylases, cellulases (EC-3.2.1.4) and finally lipases (EC-3.1.1.3) as additives in detergent formulations have become a common feature (Encarnacio´n Jurado, 2007).

During removal of glycerides by washing with alkaline solution of surfactant, fatty acids are easily removed from the fabric by formation of water soluble fatty acid soaps but triglycerides are not saponified by the alkaline solution and remain on the fabric (Thirunavukarasu a et.al 1979).

Lipases improve the washing capacity of the protease containing detergents and improve the removal of fatty food stains and sebum from fabrics which are difficult to remove under normal washing conditions (Sharma a ,*et al*).

1.4.3. Objectives of uses enzymes in laundry detergents:

Dirt comes in many forms and includes proteins, starches and lipids. In addition, clothes that have been starched must be freed of the starch. Using detergents in water at high temperatures and with vigorous mixing, it is possible to remove most types of dirt but the cost of heating the water is high and lengthy mixing or beating will shorten the life of clothing and other materials (Sarlo, Kirchner, 2002).

The use of enzymes allows lower temperatures to be employed and shorter periods of agitation are needed, often after a preliminary period of soaking. In general, enzyme detergents remove protein and lipids from clothes soiled with blood, milk, sweat, fats, oils… etc. far more effectively than non-enzyme detergents (Sarlo, Kirchner, 2002).

The enzymes used in laundry detergents act on materials that make up a variety of stains and soils so that these materials can be washed away more easily. These enzymes are named after the materials they can act upon, for example, proteases break down protein based stains, lipase break down lipid (fat) based stains and amylases break down starches and other carbohydrate based stains (amyl is Greek for starch) (Sarlo, Kirchner, 2002).

Since one enzyme molecule can act on many substrate (i.e., soil) molecules, a small amount of enzyme added to a laundry detergent can provide a big cleaning benefit to the consumer (Sarlo, Kirchner, 2002)

One of the driving forces behind the development of new enzymes or the modification of existing ones for detergents is to make enzymes more tolerant to other ingredients, e.g. builders, surfactants and bleaching chemicals, and to alkaline solutions. in general, better cleaning performance:

- Shorter washing times.
- Reduced energy consumption by enabling lowering washing temperatures.
- Reduced water consumption through more effective soil release.
- Minimal environmental impact since they are readily biodegradable and make it possible to reduce the alkali content in wash liquors.
- Environmentally friendlier wash water effluents.

The use of enzymes in detergent formulations is now common in developed countries, with over half of all detergents presently available containing enzymes. In spite of the fact that the detergent industry is the largest single market for enzymes at 25 - 30% of total sales (Ochoa-Gómez 1996).

Detergents continue to be crucially important to the quality of life worldwide. Their applications are broad, ranging from domestic cleaning products and personal hygiene to the manufacture of polymers by polymerization in emulsions (Ochoa-Gómez 1996).

An ideal detergent enzyme should be stable at high pH and temperature, withstand oxidising and chelating agents, effective at low enzyme level and have broad substrate specificity (Jurado,*et al*,2006).

1.5 Lipases Enzyme

Lipases (triacylglycerol acyl hydrolase's (E.C. 3.1.1.3)) are one of the most important classes of hydrolytic enzymes that catalyze both the hydrolysis and the synthesis of esters. Lipases have a number of unique characteristics, including substrate specificity, stereo specificity, regioselectivity and the ability to catalyze a heterogenous reaction at the interface of water soluble and water insoluble systems (Lenting, *et al*,1993).

1.5.1. Used lipase in Industry:

Microbial lipases have already established their vast potential regarding to their usage in different industries. The interest in microbial lipase production has increased in the last decades, because of its large potential in industrial applications as additives for foods (flavor modification), fine chemicals (synthesis of esters), waste water treatment (decomposition and removal of oil substances), cosmetics (removal of lipids), pharmaceuticals (digestion of oil and fats in foods), leather (removal of lipids from animal skins) and medical (blood triglyceride assay). However, the biggest market of their use is in the detergent formulation (Janaina, *et al* 2006).

1.5.2. Lipase in detergents:

Lipases can be used to enhance the removal of oily dirt's from fabrics and hard surfaces, and to increase the detergency of compact washing powders or commercial detergents, especially at low temperatures (Gillis, 1988).

When using these lipases, a high degree of soil removal is obtained in the first wash cycle. This removal can facilitate the removal of other soil substances from the fabric by surfactants, enzymes…etc., thereby improving the overall fabric appearance.

Traditionally, lipase benefits have not been observed until after multiple and a drying step was required to show wash cycles activity. Recent work has shown that lipases can be developed that show good benefits in the first wash cycle, as shown in Figure 1.5.

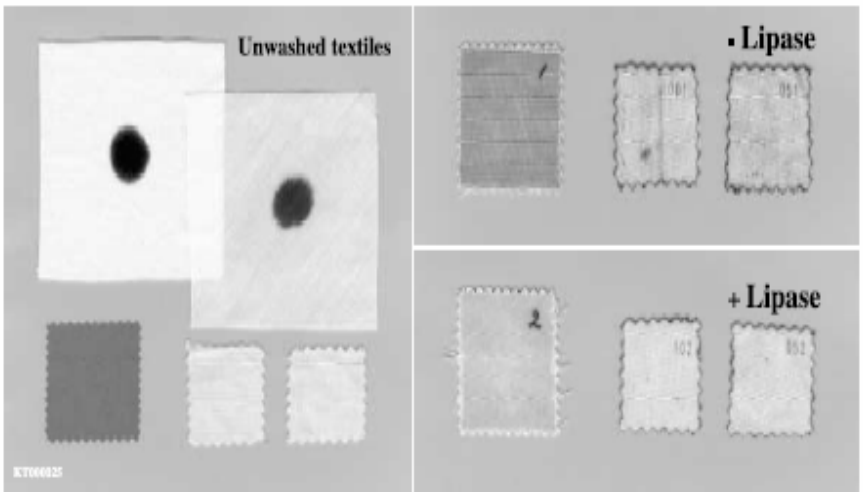


Figure 1.5: An example of in-the-wash benefits from new first-wash lipases: high stain removal of a lipstick (Reddish swatches) and reduced redeposit ion of carbon black from soiled swatches containing dirty motor oil (Large swatches in left picture) onto polyester tracer swatches (small whitish swatches Tergitometer wash). Under EU conditions with or without a first-wash lipase. (Callisen and Damhus 2000).

1.5.3. Important types of Lipase in detergents:

Table 1.4a: Main detergents Lipase Available on Market (Guy Broze 1999).

(a) Characteristics

Supplier Trade name	Production type	Optimum pH	Optimum Temp. (°C)	Characteristics (according to the supplier)
Genencor				
Lipomax®	Gene technology	8–11	50–60	High performance alkaline lipase
Lumafast				Alkaline lipase
Novo Nordisk				
Lipolase™	Gene technology	7–12	20–70	1,3-Specific lipase with broad substrate specificity

Table 1.4b: Main detergents Lipase Available on Market (Guy Broze 1999).

(b) Availability

Name	Physical form	Available concentration	Recommended dosage[a]
Lipomax CXT	Granules	1000 DLU/g	0.2–0.5% (compact)
Lumafast 2000G	Granules		
Lipolase 100T	Granules	100 KLU/g	0.2–0.6%
Lipolase 100L	Liquid	100 KLU/g	
Lipolase Ultra 50T	Granules	50 KULU/g	
Lipolase Ultra 100L	Liquid	100 KULU/g	

1.5.4. Lipase hydrolysis:

The role of lipase in detergency is to promote the removal of triglycerides by lipolytic hydrolysis to diglycerides, monoglycerides, and free fatty acids (Gillis, 1988).see Figure 1.6:

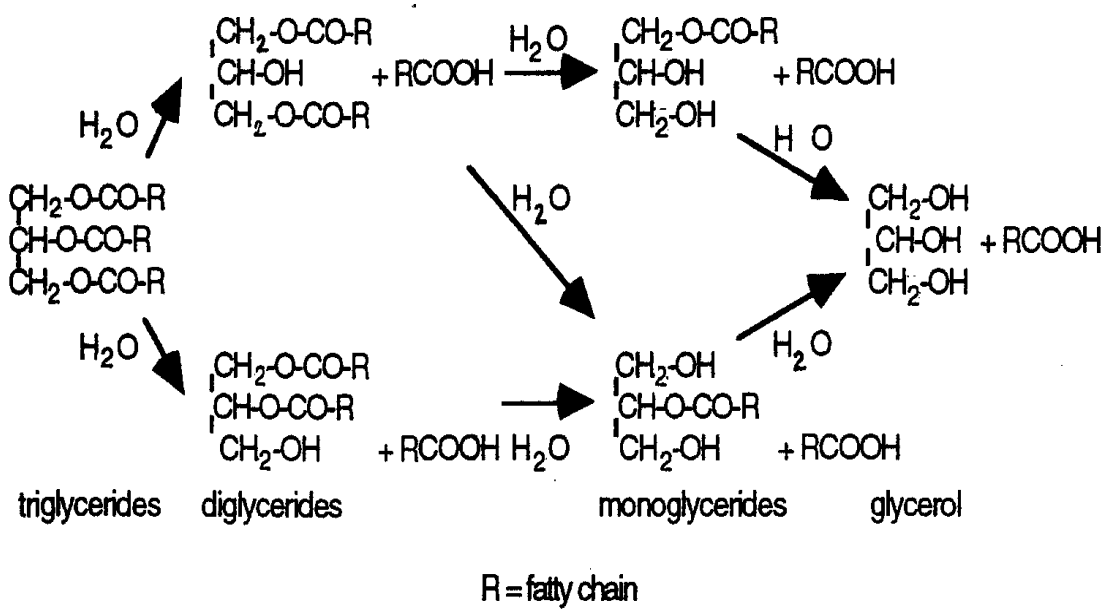


Figure 1.6: Enzymatic hydrolysis of triglyceride (Guy Broze 1999).

1.5.5. Lipase and environmental protection:

The importance of lipase in washing agents results not only from their high efficacy, but also from reasons of environmental protection. Phosphates which are used in synthetic washing agents, are known to pollute waste waters. Stringent environmental control has resulted in the use of lower levels of phosphate builders in commercial detergents thus bringing about a decrease in detergent efficiency (Davranov 1994, Haupt,1983).

A recent trend is to reduce this phosphate content for environmental reasons. It may be replaced by sodium carbonate plus extra protease.

1.6 Literature comments on lipase

Research's into the use of lipases in detergent formulas focuses primarily on the development of new enzymes with improved resistance to surfactants and better performance under practical washing conditions.

Academic research studied the activation/inhibition of lipase from pencillium cyclopium in the presence of several surfactant. They found an increase in detergency in the presence of enzyme. Hemachander and Puvanakrishnan and Fujii et al. (Jurado, *et al*, 2006), showed a 20% increase in the effectiveness of washing in systems with lipases.

Obendorf et al. (Jurado, *et al*, 2006), testing commercial lipase SP1013, observed that the hydrolysis of the triglycerides accelerated the washing process.

Detergents invariably contain surfactants and various auxiliary agents. These components may interact with lipase, resulting in activation or inhibition of its activity. From the viewpoint of anticipated increased use of lipase in the detergent industry, an understanding of the compatibility of lipase with surfactants and auxiliary agents is important. Although there are several studies on the effects of surfactants on lipases, relatively little is known about the effects of surfactants on microbial lipases (Jaeger, Reetz 1998).

The important of this study said that In general, the efficiency of stain removal by lipase depends largely on amount of soil, lipase concentration, washing conditions, and the substrate. Reported that lipase activity was strongest at 30^{o}C and was strongly inhibited by the presence of anionic surfactants (Jaeger, Reetz 1998).

The work by Kawase et al. and more recent studies by Mozaffar et al. showed that lipase activity was dependent on both type and concentration of the surfactant (J. Xia a'l et al.,1996).

Kawase et al. reported on the relationships between surfactants and lipases from the viewpoint of pH changes. They found that, while nonionic surfactants activated lipases, the effect of anionic surfactants on enzyme activity varied according to the lipase and, for the most part, was inhibitory. They indicated, however, that the inhibition of lipase by anionic surfactants could be avoided by mixing nonionic surfactants (Xia a'l et al.,1996).

Andree et al. (Jurado, *et al*, 2006), found that the use of lipases together with a nonionic surfactant led to detergency values similar to those reached at concentrations greater than those for the surfactant, revealing a synergetic effect with these surfactants.

Washing experiments with textiles conducted by Fujii et al. (Jurado, *et al*, 2006), have suggested that regardless of the type and concentration of surfactant, the addition of lipase boosts the effectiveness of the wash.

In addition, assays made with a nonionic surfactant and lipase presented higher detergency values than those made with anionic surfactants and lipase. Helistö and Korpela , Xia et al. and Andree et al. (Jurado, *et al*, 2006), found that anionic surfactants, including linear alkylbenzene sulfonate (LAS), inhibited lipases.

The use of nonionic surfactants especially alcohol ethoxylates and alcohol ethoxysulfates positively influenced the activity and stability of enzymes (Jurado, *et al*, 2006).

The lipase activity specific is maximal at pH 9.5 and 55^{o}C .(Habib Horchani, et al, 2009).

Lipases from Candida cylindracea and A. niger (Hemachander, Puvanakrishnan 2000) have been tested for their efficiency in removing olive oil from cotton fabric with an aqueous solution of lipase with or without surfactants under various environmental conditions (Hemachander, Puvanakrishnan 2000).

Tatara et al. (Hemachander, Puvanakrishnan 2000). have evaluated adaptability of various kinds of lipases in practical laundry conditions. In his study, the effect of R. pickettii lipase as an additive in detergent formulations has been evaluated in detail. (Hemachander, Puvanakrishnan 2000).

Various surfactants and detergents were first evaluated for their efficiency in removal of olive oil from cotton fabric with and without lipase. The percentage of oil removed from the fabric was higher (6.6–21.1%) in the presence of lipase for all detergents , Similar results have been reported for lipases from A. niger, C. cylindracea and R. pickettii (Thirunavukarasu et al. 1979).

Among the detergents, the most effective detergent in removing the triglycerides from the fabric and the removal of olive oil with the addition of 1000 U lipase was 21.1% higher than without lipase. (Thirunavukarasu et al. 1979.

However, only 14% of the oil was removed when washing was carried out in buffer solution and addition of lipase to the buffer solution increased the percent oil removal to 32.5% under the same conditions (Thirunavukarasu et al. 1979).

In another studies for effect lipase in washing, that removal of olive oil from cotton fabric was higher in the presence of lipase for all detergents (5-13%), and its could be used in laundry detergents to promote removal of triglyceride soils of human sebum which are difficult to remove under normal washing condition (Kamini, et al. 1997).

Last studies about the impact of lipase in laundry detergents were concluded that lipase enzyme with detergent enhances removal of oily soils at lower washing temperatures. This indicates that lipase enhanced soil removal for both soil types (Kamini, et al. 1997).

We can see some images for yarns of cotton take by microscope to promote us about the effect of lipase in removal of soils from this yarns.

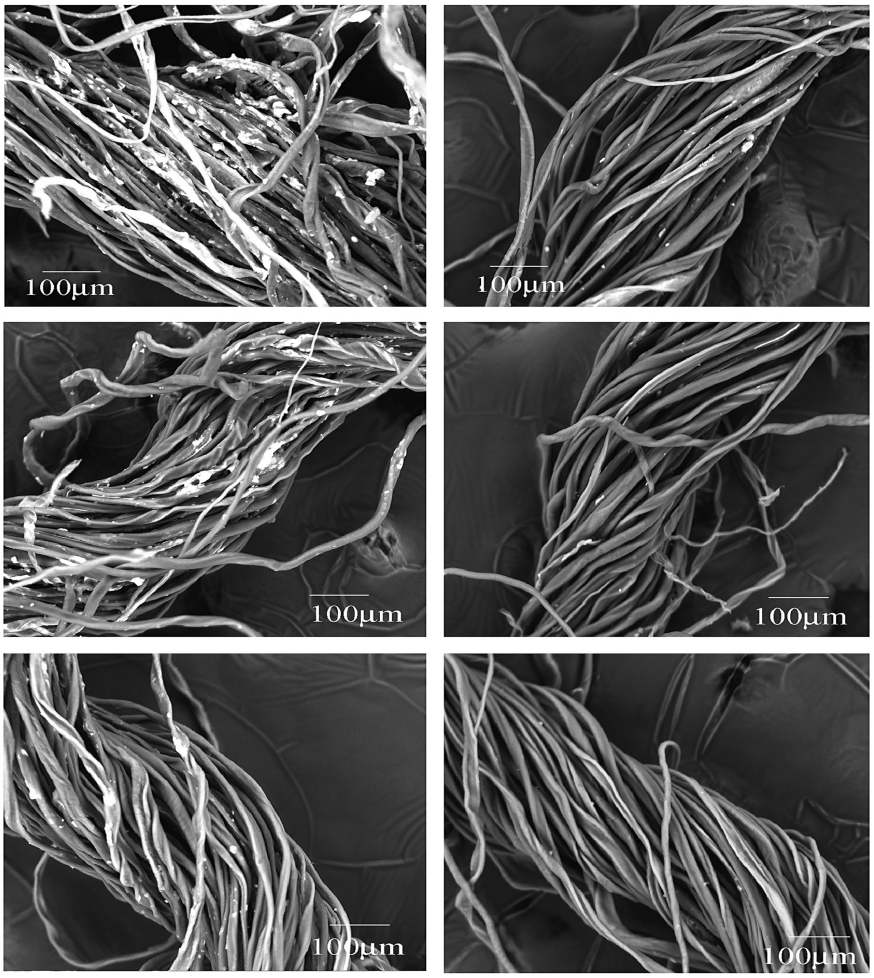


Figure 1.7: Backscattered electron images of longitudinal sections of yarn from cotton fabrics (untreated, top; mercerized, middle; carboxymethylated, bottom) that were washed with lipase (left) and without lipase (right).(Obendorf*a,et* al. 2001).

As most of the industrial processes operate at a temperature exceeding 45°C, lipase should be active and stable at a temperature of around 50°C.

From the previous references, we can note that more studies determine the optimum PH between 7 and 10, the optimum temperature between 45°C - 55 °C for activity of lipase in detergents, and this ranges returns to the different sources of lipase enzyme.

1.7 Problem

Washing clothes from hard oily stains, the high cost of surfactant and high cost of heating water used in laundry detergents are the biggest problems of laundry detergents.

1.8 Purpose of this study

The purpose of this study is to remove the hard oily stains from clothes with lower percentage mass of surfactant and the heating temperature of water by using alkaline lipase enzymes (Lipolase 100T) with ionic and non ionic surfactant like LAS and NP6 respectively, and to determine the optimum temperature and pH for alkaline Lipolase 100T activity in laundry detergents washing solution.

Part Two

Experimental Part

2. Introduction

The study seeks to determine activity and effectiveness for one kind of enzyme called Lipolase 100 T. and this by using olive oil as substrate. The study used two methods for the analysis, one is based on determining the proportion of fatty acids resulting from the hydrolysis of olive oil by enzyme, and this leads to a reduction in the degree of pH of the analysis material in various conditions such as temperature, concentration of surfactants, pH medium and concentration of enzyme. Then conclusion analysis results to determine optimal conditions for the washing solution that can be used in laundry detergents.

Another method of analysis is to choose the optimum solutions from the first method of analysis and washing different pieces of clothing such as cotton, wool and poly ester using a model of washing machine have been designed specifically for this study, and determine the effectiveness of this solutions on laundry detergent.

There are many methods to determine the lipolase activity that use in the different studies, and there is many machines or devices that used to washing pieces of clothes or any substrate used to determine the activity of lipase, this methods depends on the composition of solution.

2.1 Three methods to determine lipase activity

Three methods are generally used to determine lipase activity, these methods are (Guy Broze 1999):

- **PH stat Method**: As the lipase catalyzed fat splitting reaction proceeds, the pH of the reaction medium goes down because of the fatty acid released, and by adding an alkali we can determine the concentration of fatty acid, this method is rapid and easy.

- **The free fatty acid concentration Method**: this method also be determines lipase activity by titrating the reaction medium after the end of digestion.

- **DLU Method**: This method is based on the lipase catalyzed hydrolysis of 1,2-o-dilauryl-rac-glycerol-3-glutaric acid resorufin ester at pH 6.8 and 37°C, A chromogenic groups released and the color intensity is determined by spectrophotometry.

2.1.1. Devices for washing substrate:

There are many devices used to washing pieces clothes in these studies like " Bath-Substrate-Flow(BSF)" device with full description, Figure 2.1(Jurado-Alameda et al 2003). And this device can be used to study the dynamics of washing.

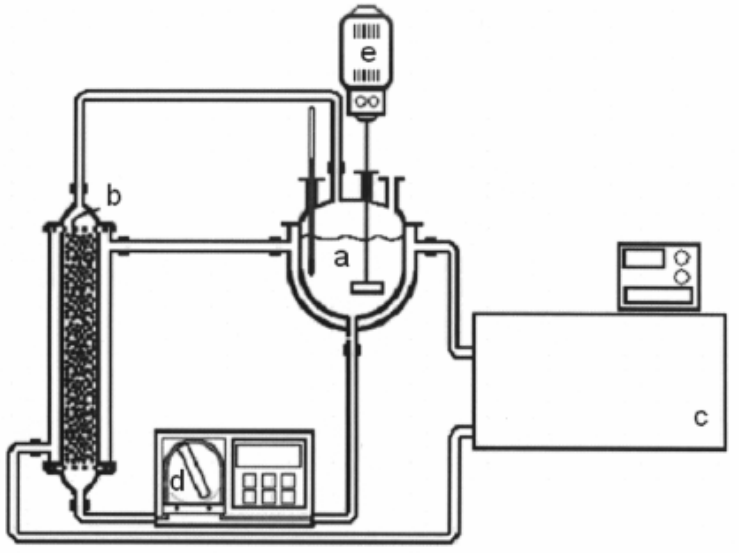


Figure 2.1 A simplified scheme of washing device " bath–substrate –flow (BSF)" with full description of the same can be found elsewhere Once soiled, the substrate, composed of borosilicate glass beads 3 mm in diameter, is placed inside a column (B). The washing solution flows upward from the tank (A) toward the column, driven by a peristaltic pump (C). A constant temperature is maintained throughout the system by circulating water from the thermostatic bath (D). Therefore, the soiling agent is removed from the substrate by means of the flow of the washing solution, which contains the components of which the detersive performance is being tested (Jurado-Alameda et al 2003).

In this study a simple washing device was designed by using the principle of washing machine used in our homes, but in a small volume, Figure 2.2(a,b) describe this device.

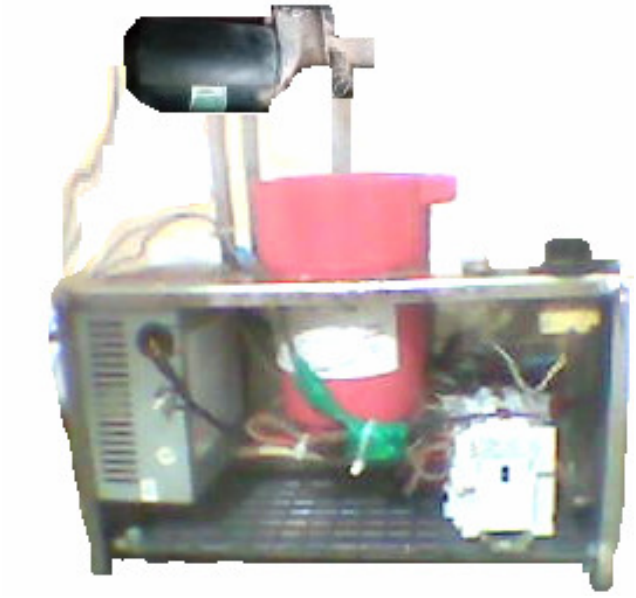


Figure 2.2a: A simple design of the washing device used in this study.

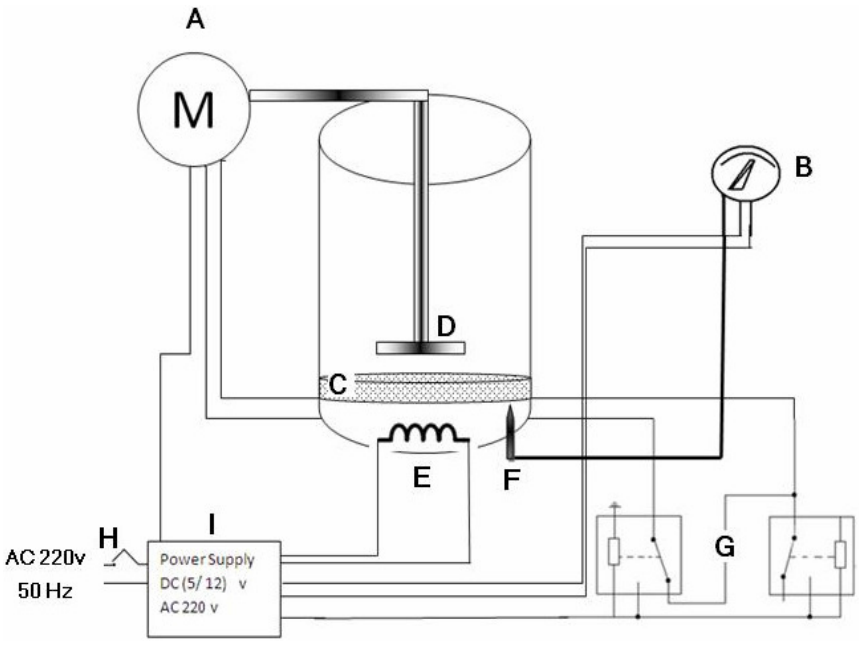


Figure 2.2b: A simplified scheme of the washing device used in this study where (A) is motor (B) thermostat, (C) refinery, (D) mixer, (E) heater, (F) sensor of thermostat, (G) conductor, (H) switch, (I) power Supply.

2.2 Materials and reagents

Table 2.1: Material and reagents used in this study.

Materials	Source
Enzyme Lipolase 100T	Novozymes-Denmark
Anionic surfactant (LAS)	Al-bareeq company
Nonionic surfactant (NP6)	Al-bareeq company
NaOH	Frutarom
Olive Oil	Ramallah - Palestine
Ethanol 96%	Pharamco-USA
Indicator	Sigma Aldrich
Distilled water	Al-Quds university lab

2.2.1. Information about Materials and reagents:

2.2.1.1. Lipolase enzyme:

The first commercially successful lipase was introduced by Novo Nor disk A/S in 1988 under the trade name of Lipolase, wide range of lipases are known. The first industrial lipase was produced by recombinant DNA technology, has been produced by transformation of a fungal lipase gene into an Aspergillus strain. Other formulations such as Lipomax produced by Gist-Brocades have Pseudomonas lipases (Gillis, 1988).

Lipolase is active and stable under alkaline conditions and over a broad temperature range, which is essential for enzymes in detergents (Gillis, 1988).

Lipases break down triglycerides into their component glycerol and fatty acid units thereby increasing their water solubility. Owing to their hydrophobicity, these stains are removing via the conventional surfactant among the most difficult to technology commonly found in detergents (Gillis, 1988).

2.2.1.2. Linear alkylbenzene sulfonate (LAS):

LAS is an ionic surfactant – see chemical structure in Figure 2.3 - that used in most of detergents to lowering the surface tension of water to help removing the soils from fabrics.

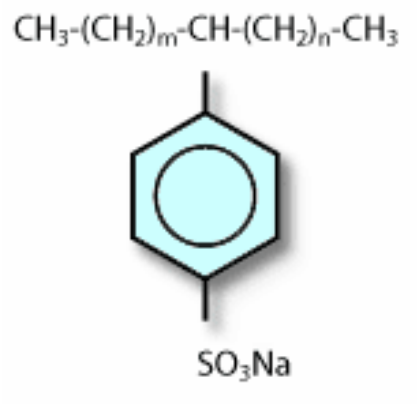


Figure 2.3: chemical structure of (LAS) (lasinfo.org 2009).

LAS Surfactants have characteristics like to help the fabrics get wet. They also keep the removed soil in suspension during the washing process in order to be washed away with the suds.

LAS is the safest one from an environmental and human health point of view. In fact, after more than 30 years of world-wide use, no environmental or human safety negative impact has ever been identified for LAS (lasinfo.org 2009).

2.2.1.3. Nonoxynol or Nonyl Phenol Ethoxylate 6 (NP6):

Nonylphenol ethoxylate (6) is nonionic surfactant - see chemical structure in Figure 2.4 - Its used as detergents, emulsifiers, wetting agents, defoaming agents….etc.

Nonylphenol ethoxylates compounds are synthesised by alkylation of phenol by a trimer of propene under the influence of an acidic catalyst.

Since these compounds are widely produced over the world and are designed to flush down the sink, concerns about their safety has increased in the past years. Several research programs

have shown that these surfactants have a mild to medium estrogenic function. Consequently, this class of detergents has been effectively banned for commercial "down-the-drain" applications in Europe, and these compounds are not found in laundry detergents in the USA (en.wikipedia.org 8.3.2009).

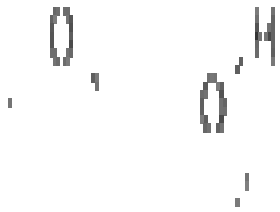


Figure 2.4: Chemical structure of Nonyl Phenol Ethoxylates (en.wikipedia.org8.3.2009)

Table 2.2: Tools and equipment used in this study.

Tools	**Source / model**
PH Meter	Mettler Toledo
Washing device	Locally made
Glasses	Pyrex
Cylinders (100 , 500 , 1000 ml)	Pyrex
Water Bath	Gas furnace
Thermometer	Al-Quds university lab
Pipits (2.5, 5, 10 ml)	Latex free romed - Holland
Burette (50 ml)	Pyrex
Balance (4 digital)	Ramco AB104

2.3 The procedure of work

The procedures of the study are divided into two parts:

Part one: To determine the optimum conditions for lipolase activity that include various concentrations of surfactant and enzymes at several pH values and various temperature, and that by using Analytical method of Kawase et al. with some modification (Xia[a'l] et al. 1996).

Part two: Washing pieces of different types of clothes like cotton, wool, poly ester ...ect. by using optimum solutions that we have from part one.

2.3.1. The procedure of part one:

Section A: (Preparing raw material for study)

1. Different concentrations (1,0.5,0.1 g/L) of Lipolase 100T are prepared by dissolving it in distilled water.
2. Aqueous solutions with different concentrations (5,1,0.5 g/L) of anionic surfactant (LAS) and nonionic surfactant (NP6) are prepared.
3. Nine solutions are prepared that contain different types of surfactants and pH medium as it is shown in Tables 2.3(a,b,c).
4. The above solutions are adjusted to the desired pH needed (7,8.5,10) in different stages.

Table 2.3: Nine solutions of surfactants and distilled water are adjusted to the desired pH (7,8.5,10).

No. sample	H_2O (mL)	LAS sol'n (mL)	NP6 sol'n (mL)
1	8.5	0.0	0.0
2	7.0	2.5	0.0
3	6.0	2.5	0.0
4	7.0	0.0	2.5
5	6.0	0.0	2.5
6	4.5	2.5	2.5
7	3.5	2.5	2.5
8	3.5	1.5	3.5
9	3.5	3.5	1.5

Section B: (assay of lipolase (100T) activity)

1. Nine samples containing H_2O, LAS, NP6, and olive oil are prepared.(see Table 2.4):

Table 2.4 Preparing samples from one to nine:

No. sample	H_2O (mL)	LAS sol'n (mL)	NP6 sol'n (mL)	Olive oil (mL)
1	8.5	0.0	0.0	0.5
2	7.0	2.5	0.0	0.5
3	6.0	2.5	0.0	0.5
4	7.0	0.0	2.5	0.5
5	6.0	0.0	2.5	0.5
6	4.5	2.5	2.5	0.5
7	3.5	2.5	2.5	0.5
8	3.5	1.5	3.5	0.5
9	3.5	3.5	1.5	0.5

2. The samples are introduced in water bath for five minutes until the desired temperature is reached.
3. One ml of Lipolase 100T solution is added to samples No. (1,3,5,7,8,9) - see Table 2.3 - and slowly manual shaking for 20 mint. This step is repeated with the three concentrations of Lipolase 100T.

Table 2.5: The final composition of samples put in water bath to assay lipase activity.

No. sample	H_2O (mL)	LAS sol'n (mL)	NP6 sol'n (mL)	Olive oil (mL)	Lipase sol'n (mL)
1	8.5	0.0	0.0	0.5	1.0
2	7.0	2.5	0.0	0.5	0.0
3	6.0	2.5	0.0	0.5	1.0
4	7.0	0.0	2.5	0.5	0.0
5	6.0	0.0	2.5	0.5	1.0
6	4.5	2.5	2.5	0.5	0.0
7	3.5	2.5	2.5	0.5	1.0
8	3.5	1.5	3.5	0.5	1.0
9	3.5	3.5	1.5	0.5	1.0

4. After 20 min, 12 mL of 96% ethanol are added to every sample to stop the activity of the enzyme.
5. The fatty acid content in the mixture was determined by titration to the end point with 0.0045 M NaOH using indicator.
6. The Blank take as the same assay procedure by adding the enzyme after addition ethanol.

Each sample from these (Table 2.5) is put to different conditions (pH, temp., Lipolase and surfactant concentrations).

7. All results are recorded in the previous Tables for later analysis.

2.3.2. The procedure of part two:

In this part, pieces of different types of cloth are washed by using the ten optimum samples of that assay in part one by using the washing device as follows:

1. Washing solutions are prepared as in Table 2.5 for the ten optimum samples assayed by multiplied by 50 to give 500 ml washing solution.
2. The clothes are soiled with one ml of olive oil.
3. The washing solution is placed in the washing device.
4. The substrates are placed in the washing device and washed for 20 min.
5. The pieces of cloth are removed from the washing device and washed them twice with distilled water.
6. The washed pieces are examined by taking high resolution images for these pieces.
7. All the results are recorded in the later analysis.

2.4 Lipolase activity equation

The following equation was used to determine Lipase activity (Xia [a'l], et al. 1996):

$$\text{Unit/mL} = \frac{(\text{S-B})4.5f}{(t)(V)}$$

Where:

S: Volume (mL) of 0.0045 M NaOH to titrate lipolase hydrolyses.

B: Volume (mL) of 0.0045 M NaOH to titrate blank solution.

f : Dilution factor for lipolase solution.

4.5 : µmole of NaOH contained in 1 mL 0.0045 M NaOH solution.

t : Reaction time.

v : Volume (mL) of the lipolase solution added to the assay mixture.

One unit of the activity is defined as: The amount of enzyme that liberate one µmole equivalent of fatty acid from olive oil in one mint under the analytical condition.

Part Three

Results and Discussion

3. Introduction

The process of analyzing the results of this study is divided into two parts:

The first part is to study the effect of surfactants on the effectiveness of the enzyme , and that by chart the relative activity of lipolase 100T - Unit/mL - with samples that have been studied (1-9) which contain different types and concentration of surfactants (0.5, 0.1, 0.05) ,see the general model for this relationship in Figure 3.1.

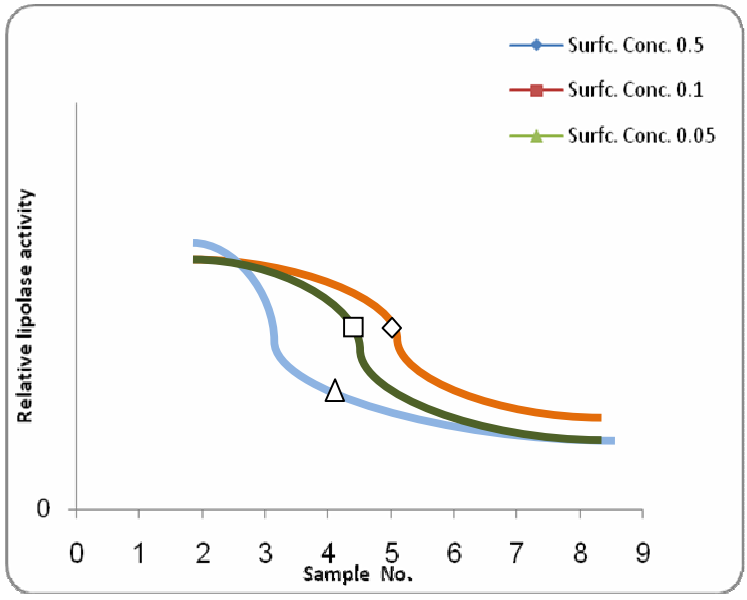


Figure 3.1: The general model for relative enzyme activity and surfactants concentration relationship.

The charts are repeated with different enzyme concentrations (0.1, 0.05 and 0.05) and temperatures (40 ^{o}C, 50 ^{o}C and 60^{o}C). this will be clear in later Tables and charts.

Each of the above charts will also be repeated with different pH mediums (7, 8.5, 10).

The second section of the analysis, includes the laundering pieces of cloth by the best solutions obtained from this study. High resolution images of these pieces were taken to display the output of the cleaning solutions.

3.1 Analysis of section one

The analysis equation was used to calculate results with this variables:

(S-B): Volumes of NaOH are calculated. see Tables (3.1, 3.5, 3.9).

f : Dilution factor equal total volume of sample (10 mL) divided by one (volume of lipolase solution used in analysis), so the dilution factor is 10.

t : Reaction time equal 20 minute.

V : Volume of lipolase used in analysis equal one mL.

After the application of data obtained within the equation, the subsequent table shows the result.

The samples (2,4,6), which do not contain the enzyme do not show a significant change in all the results of the analysis with all the changes that have happened to all the other samples, so they are away from the comparison and analysis of the other results for samples.

3.2.1. Results analysis at PH 7:

Volumes of NaOH are calculated for **Lipolase 0.1 concentrations** (in samples) with different surfactant concentrations that is clear in later Tables 3.1(a,b,c):

Table 3.1a: Volumes of NaOH at **Lipolase 0.1 concentrations** with different temperature and surfactant concentrations**:**

	Surfactant 0.5 conc.			**Surfactant 0.1 conc.**			**Surfactant 0.05 conc.**		
S No./T*	**40°C**	**50°C**	**60°C**	**40°C**	**50°C**	**60°C**	**40°C**	**50°C**	**60°C**
1	2.9	3.3	3.9	2.9	3.3	3.9	2.9	3.3	3.9
3	0.5	0.5	0.6	0.4	0.6	0.8	0.5	0.6	0.8
5	1.4	2.0	2.3	0.7	1.1	1.5	0.5	0.5	0.9
7	0.5	0.6	0.7	0.5	0.7	0.9	0.4	0.6	0.8
8	0.4	0.5	0.6	0.3	0.5	0.7	0.4	0.6	0.7
9	0.8	0.9	1.1	0.4	0.6	0.8	0.6	0.6	1.1

*sample Number / Temperature

Table 3.1b: Volumes of NaOH at **Lipolase 0.05 concentrations** with different temperature and surfactant concentrations**:**

	Surfactant 0.5 Conc.			**Surfactant 0.1 Conc.**			**Surfactant 0.05 Conc.**		
S No./ T	**40C°**	**50C°**	**60C°**	**40C°**	**50C°**	**60C°**	**40C°**	**50C°**	**60C°**
1	1.9	2.4	2.8	1.9	2.4	2.8	1.9	2.4	2.8
3	0.3	0.4	0.5	0.2	0.3	0.4	0.3	0.4	0.5
5	0.9	1.3	1.5	0.3	0.6	1.1	0.2	0.3	0.5
7	0.6	0.7	0.8	0.4	0.6	0.1	0.2	0.3	0.2
8	0.5	0.6	0.8	0.2	0.3	0.4	0.4	0.2	0.5
9	0.8	0.9	1.1	0.2	0.3	0.5	0.4	0.5	0.7

Table 3.1c: Volumes of NaOH at **Lipolase 0.01 concentrations** with different temperature and surfactant concentrations**:**

	Surfactant 0.5 conc.			**Surfactant 0.1 conc.**			**Surfactant 0.05 conc.**		
S No./ T	**40°C**	**50°C**	**60°C**	**40°C**	**50°C**	**60°C**	**40°C**	**50°C**	**60°C**
1	0.7	0.9	1.2	0.7	0.9	1.2	0.7	0.9	1.2
3	0.1	0.1	0.3	0.1	0.2	0.4	0.5	0.1	0.4
5	0.5	0.6	0.7	0.3	0.5	0.6	0.2	0.5	0.7
7	0.2	0.3	0.5	0.4	0.5	0.6	0.1	0.2	0.3
8	0.1	0.2	0.3	0.2	0.3	0.6	0.1	0.3	0.5
9	0.5	0.6	0.8	0.5	0.6	0.7	0.3	0.4	0.6

The results obtained were multiplied by ten to give a better comparison of the activity of the enzyme.

3.2.1.1. Charts for results analysis at PH 7:

Tables of Lipolase (100T) activity (Unit/mL) at different temperature and Surfactant concentrations and their charts:

- First at **Lipolase (100T) 0.1 concentration** with different surfactant concentrations. and this clear in Tables 3.3(a,b,c) and Figures 3.3(a,b,c).

Table 3.2a: Lipolase(100T) activity (Unit/mL) at 40°C

	Temp. 40°C		
Sample No.	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	65.3	65.3	65.3
3	11.3	9.00	11.3
5	31.5	15.8	11.3
7	11.3	11.3	9.00
8	9.00	6.75	9.00
9	18.0	9.00	13.5

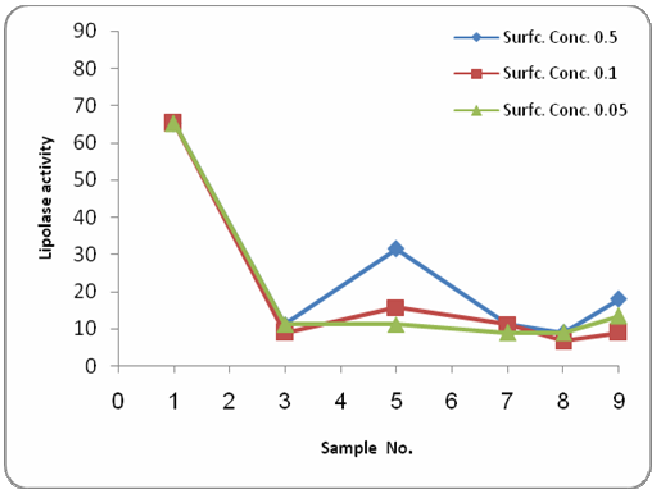


Figure 3.2a: Chart of Lipolase activity at 40°C and different surfactant concentrations.

Table 3.2b: Lipolase(100T) activity (Unit/mL) at 50°C

Sample No.	Temp. 50°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	74.3	74.3	74.3
3	11.3	13.5	13.5
5	45.0	24.8	11.3
7	13.5	15.8	13.5
8	11.3	11.3	13.5
9	20.3	13.5	13.5

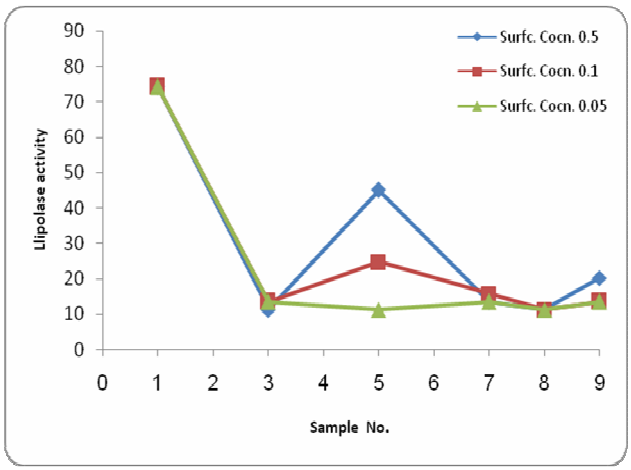


Figure 3.2b: Chart of Lipolase activity at 50°C and different surfactant concentrations.

Table 3.2c: Lipolase(100T) activity (Unit/mL) at 60°C

Sample No.	Temp. 60°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	87.8	87.8	87.8
3	13.5	18.0	18.0
5	51.3	33.3	20.3
7	15.8	20.3	18.0
8	13.5	15.8	15.8
9	24.8	18.0	24.8

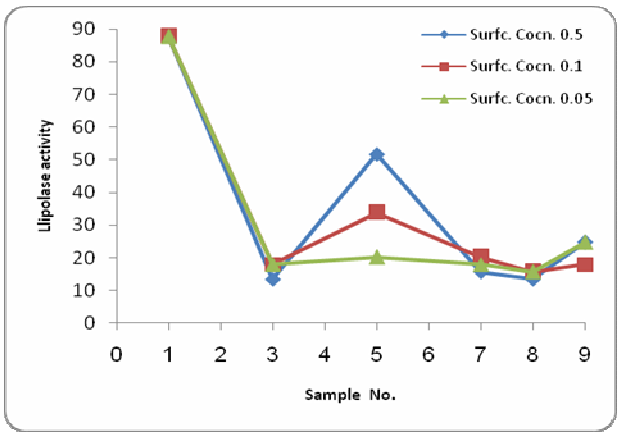


Figure 3.2c: Chart of Lipolase activity at 60°C and different surfactant concentrations.

- Second: At **Lipolase (100T) 0.05 concentration** with different surfactant concentrations, and this clear in Tables 3.3(a,b,c) and Figures 3.3(a,b,c).

Table 3.3a: Lipolase(100T) activity (Unit/mL) at 40°C

Sample No.	Temp. 40°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	42.8	42.8	42.8
3	6.75	4.50	6.75
5	20.3	6.75	4.50
7	13.5	9.00	4.50
8	11.3	4.50	9.00
9	18.0	4.50	9.00

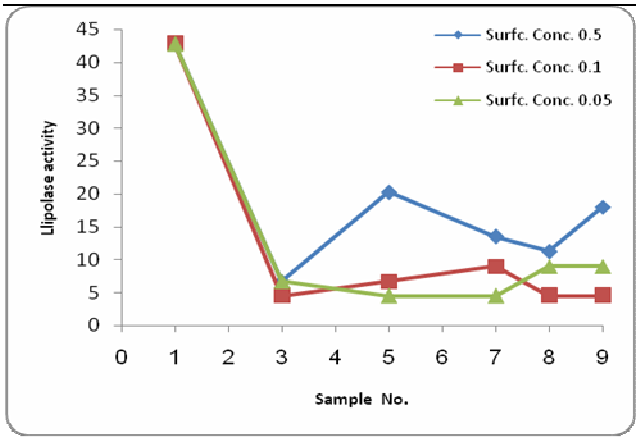


Figure 3.3a: Charts of Lipolase activity at 40°C and different surfactant concentrations

Table 3.3b: Lipolase(100T) activity (Unit/mL) at 50°C

Sample No.	Temp. 50°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	54.0	54.0	54.0
3	9.00	6.75	9.00
5	29.0	13.5	6.75
7	16.0	13.5	6.75
8	14.0	6.75	4.50
9	20.0	6.75	11.3

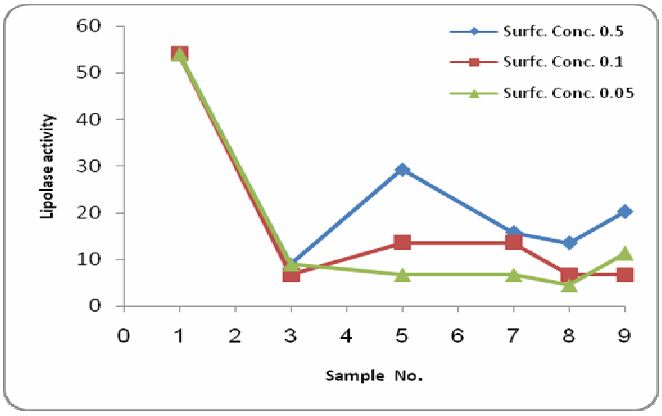


Figure 3.3b: Chart of Lipolase activity at 50°C and different surfactant concentrations.

Table 3.3c: Lipolase(100T) activity (Unit/mL) at 60°C

Sample No.	Temp. 60°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	63.0	63.0	63.0
3	11.3	9.00	11.3
5	33.8	24.8	11.3
7	18.0	2.25	4.50
8	18.0	9.00	11.3
9	24.8	11.3	15.8

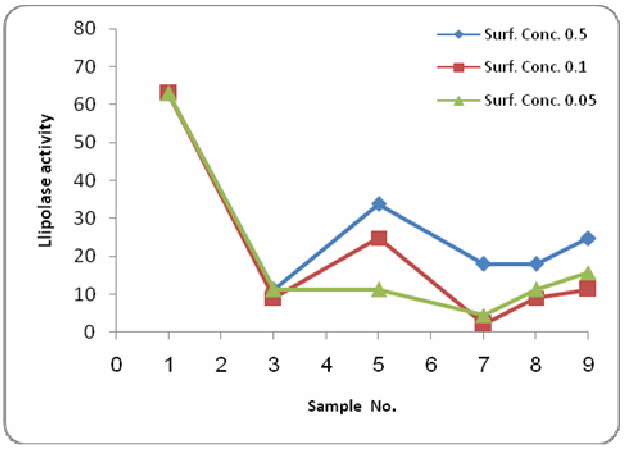


Figure 3.3c: Chart of Lipolase activity at 60°C and different surfactant concentrations

- Third: at **Lipolase (100T) 0.01 concentration** with different surfactant concentrations and this clear in Tables 3.4(a,b,c) and Figures 3.4(a,b,c).

Table 3.4a: Lipolase(100T) activity (Unit/mL) at 40°C

Sample No.	Temp. 40°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	15.8	15.8	15.8
3	2.25	2.25	11.3
5	11.3	6.75	4.50
7	4.50	9.00	2.25
8	2.25	4.50	2.25
9	11.3	11.3	6.75

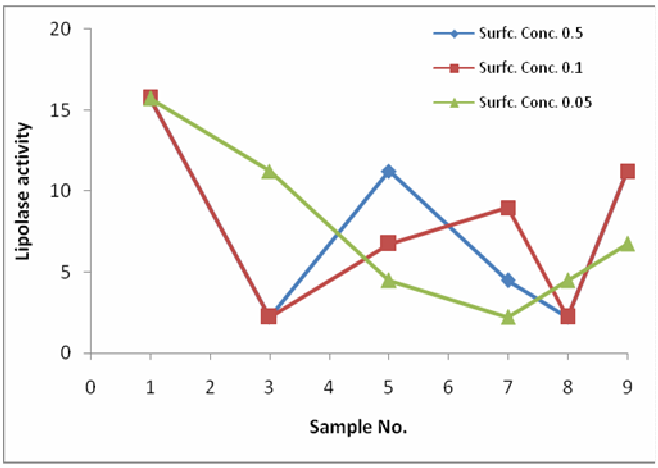


Figure 3.4a: Chart of Lipolase activity at 40°C and different surfactant concentrations

Table 3.4b: Lipolase(100T) activity (Unit/mL) at 50°C

Sample No.	Temp. 50°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	20.3	20.3	20.3
3	2.25	4.50	2.25
5	13.5	11.3	11.3
7	6.75	11.3	4.50
8	4.50	6.75	6.30
9	13.5	13.5	9.00

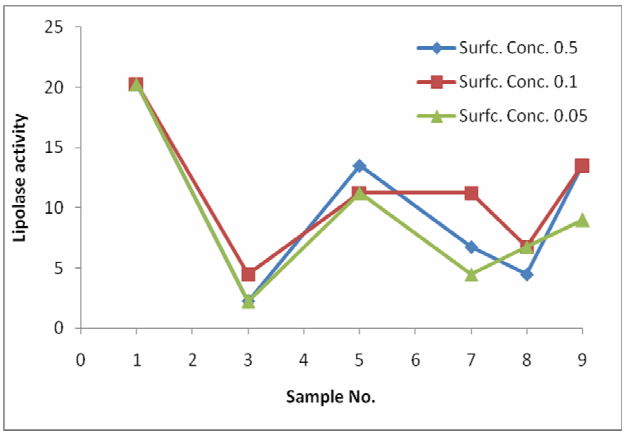


Figure 3.4b: Chart of Lipolase activity at 50°C and different surfactant concentrations

Table 3.4c: Lipolase(100T) activity (Unit/mL) at 60°C

Sample No.	Temp. 60°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	27.0	27.0	27.0
3	6.75	9.00	9.00
5	15.8	13.5	15.8
7	11.3	13.5	6.75
8	6.75	13.5	11.3
9	18.0	15.8	13.5

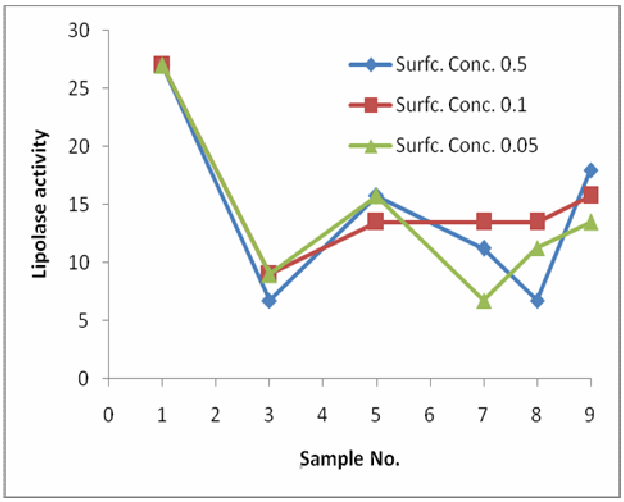


Figure 3.4c: Chart of Lipolase activity at 60°C and different surfactant concentrations

The results analyzed showed significant activity of the enzyme at pH 7 and without surfactant. The activity decreases by reducing the concentration of the enzyme in the similar samples. This is evident in all the different situations and circumstances in general.

It is also clear that the high temperature for the same conditions of samples increases the effectiveness of the enzyme.

On the other hand, the ionic surfactant LAS that was used in this study reduces the effectiveness of the enzyme when it is present in the samples was clear - and this is explained in previous studies. Other nonionic surfactants NP6 also used in this study, work to reduce the effectiveness of the enzyme, but less than LAS. This is clear in the given Tables and graphs 3.2 until 3.4.

Accordingly, the optimum effectiveness of the enzyme in this medium at temperature of 60C^{o} and the highest concentration of enzyme 0.1g/L, and then the optimum activity go to the samples contain the highest concentration of NP6 at the highest temperature (note the chart 3.2c**.**

3.2.2. Results analysis at PH 8.5:

Volumes of NaOH are calculated for **Lipolase 0.1 concentrations** with different surfactant concentrations that is clear in the later Tables 3.5(a,b,c):

Table 3.5a: Volumes of NaOH at **Lipolase 0.1 concentrations** with different temperature and surfactant concentrations**:**

	surfactant 0.5 Conc.			**surfactant 0.1 Conc.**			**surfactant 0.05 Conc.**		
S No./ T	**40^{o}C**	**50^{o}C**	**60^{o}C**	**40^{o}C**	**50^{o}C**	**60^{o}C**	**40^{o}C**	**50^{o}C**	**60^{o}C**
1	1.5	2.7	3.3	1.5	2.7	3.3	1.5	2.7	3.3
3	0.1	0.2	0.2	0.3	0.3	0.4	0.5	0.6	0.7
5	0.8	1.0	1.5	0.4	0.5	1.3	0.2	0.8	1.1
7	0.1	0.2	0.5	0.3	0.4	0.5	1.0	0.3	1.2
8	0.1	0.2	0.3	0.2	0.5	0.5	0.4	0.6	0.7
9	0.7	0.3	0.2	0.7	0.6	0.8	0.4	0.6	0.9

Table 3.5b: Volumes of NaOH at **Lipolase 0.05 concentrations** with different temperature and surfactant concentrations**:**

	surfactant 0.5 conc.			**surfactant 0.1 conc.**			**surfactant 0.05 conc.**		
S No./ T	**40°C**	**50°C**	**60°C**	**40°C**	**50°C**	**60°C**	**40°C**	**50°C**	**60°C**
1	0.9	1.9	2.6	0.9	1.9	2.6	0.9	1.9	2.6
3	0.1	0.2	0.3	0.2	0.4	0.4	0.2	0.4	0.5
5	0.3	0.4	0.6	0.4	0.6	1.0	0.9	0.5	2.4
7	0.2	0.3	0.4	0.2	0.3	0.4	0.1	0.4	0.7
8	0.1	0.3	0.3	0.1	0.2	0.3	0.3	0.4	0.5
9	0.3	0.4	0.5	0.3	0.4	0.6	0.2	0.4	0.6

Table 3.5c: Volumes of NaOH at **Lipolase 0.01 concentrations** with different temperature and surfactant concentrations**:**

	surfactant 0.5 conc.			**surfactant 0.1 conc.**			**surfactant 0.05 conc.**		
S No./ T	**40°C**	**50°C**	**60°C**	**40°C**	**50°C**	**60°C**	**40°C**	**50°C**	**60°C**
1	0.60	1.00	1.40	0.60	1.00	1.40	0.60	0.10	1.40
3	0.05	0.10	0.20	0.10	0.20	0.30	0.20	0.30	0.50
5	0.10	0.20	0.40	0.05	0.10	0.30	0.05	0.10	0.20
7	0.05	0.10	0.20	0.10	0.20	0.30	0.20	0.30	0.40
8	0.05	0.10	0.20	0.10	0.20	0.50	0.10	0.20	0.30
9	0.20	0.30	0.40	0.30	0.40	0.70	0.10	0.30	0.40

3.2.2.1. Charts for results analysis at PH 8.5:

Tables of Lipolase(100T) activity (Unit/mL) at different temperature and Surfactant concentrations and their charts:

- First at **Lipolase (100T) 0.1 concentration** and different surfactant concentrations, and this clear in Tables 3.6(a,b,c) and Figures 3.6(a,b,c).

Table 3.6a: Lipolase(100T) activity (Unit/mL) at 40°C

	Temp. 40°C		
Sample No.	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	33.8	33.8	33.8
3	2.25	6.75	11.3
5	18.0	9.00	4.50
7	2.25	6.75	22.5
8	2.25	4.50	9.00
9	15.8	15.8	9.00

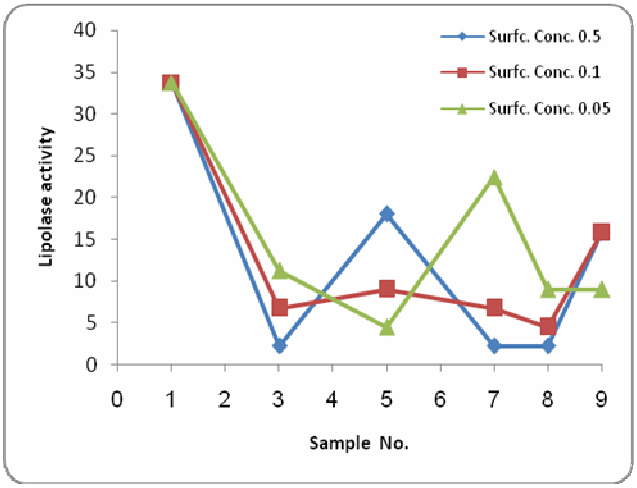


Figure 3.6a: Chart of Lipolase activity at 40°C and different surfactant concentrations

Table 3.6b: Lipolase(100T) activity (Unit/mL) at 50°C

	Temp. 50°C		
Sample No.	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	60.8	60.8	60.8
3	4.50	6.75	13.5
5	22.5	11.3	18.0
7	4.50	9.00	6.75
8	4.50	11.3	13.5
9	6.75	13.5	13.5

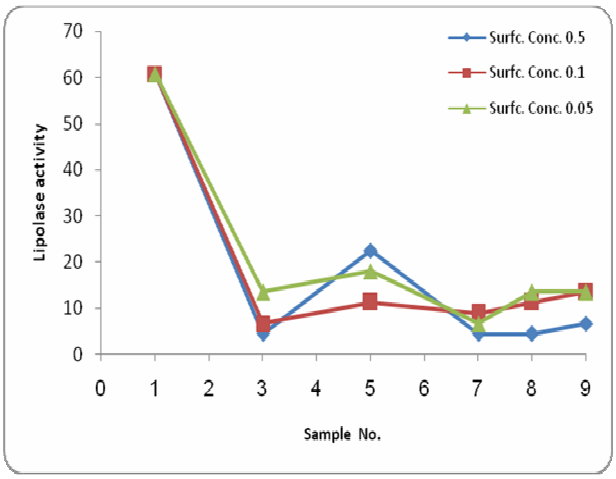


Figure 3.6b: Chart of Lipolase activity at 50°C and different surfactant concentrations.

Table 3.6c: Lipolase(100T) activity (Unit/mL) at 60°C

Sample No.	Temp. 60°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	74.3	74.3	74.3
3	4.50	9.00	15.8
5	33.8	29.3	24.8
7	11.3	11.3	27.0
8	6.75	11.3	15.8
9	4.50	18.0	20.3

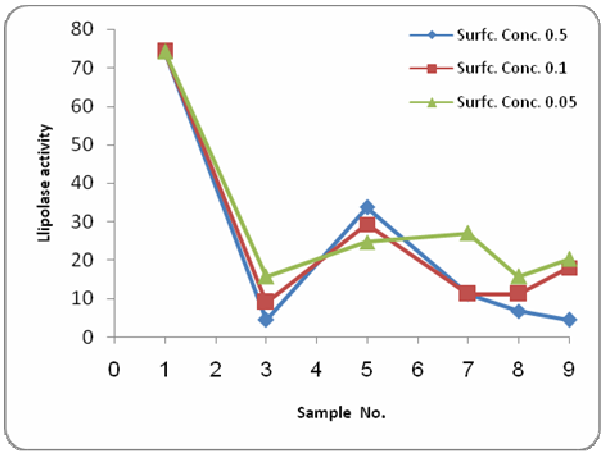


Figure 3.6c: Chart of Lipolase activity at 60°C and different surfactant concentrations

- Second: At **Lipolase (100T) 0.05 concentration** and different surfactant concentrations, and this clear in Tables 3.7(a,b,c) and Figures 3.7(a,b,c).

Table 3.7a: Lipolase(100T) activity (Unit/mL) at 40°C

Sample No.	Temp. 40°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	20.3	20.3	20.3
3	2.25	4.50	4.50
5	6.75	9.00	20.3
7	4.50	4.50	2.25
8	2.25	2.25	6.75
9	6.75	6.75	4.50

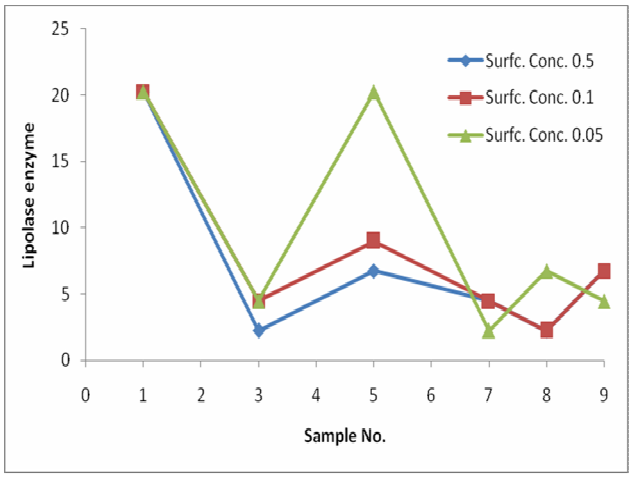


Figure 3.7a: Chart of Lipolase activity at 40°C and different surfactant concentrations

Table 3.7b: Lipolase(100T) activity (Unit/mL) at 50°C

Sample No.	Temp. 50°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	42.8	42.8	42.8
3	4.50	9.00	9.00
5	9.00	13.5	11.3
7	6.75	6.75	9.00
8	6.75	4.50	9.00
9	9.00	9.00	9.00

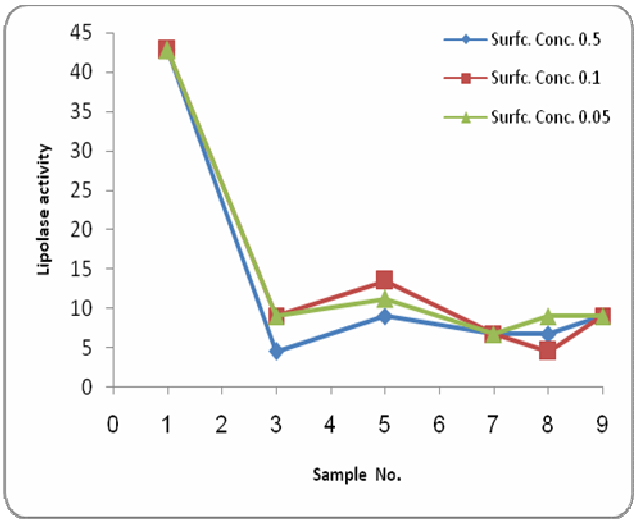


Figure 3.7b: Charts of Lipolase activity at 50°C and different surfactant concentrations

Table 3.7c: Lipolase(100T) activity (Unit/mL) at 60°C

Sample No.	Temp. 60°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	58.5	58.5	58.5
3	6.75	9.00	11.3
5	13.5	22.5	32.0
7	9.0	9.00	15.8
8	6.75	6.75	11.3
9	11.3	13.5	13.5

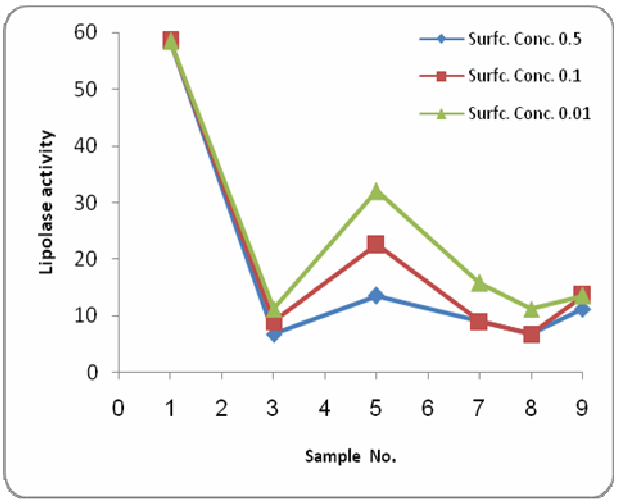


Figure 3.7c: Charts of Lipolase activity at 60°C and different surfactant concentrations

- Third: At **Lipolase (100T) 0.01 concentration** and different surfactant concentrations, and this clear in Table 3.8(a,b,c) and Figure 3.8(a,b,c).

Table 3.8a: Lipolase(100T) activity (Unit/mL) at 40°C

	Temp. 40C°		
Sample No.	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	13.5	13.5	13.5
3	1.10	2.25	4.50
5	2.25	1.10	1.10
7	1.10	2.25	4.50
8	1.10	2.25	2.25
9	4.50	6.75	2.25

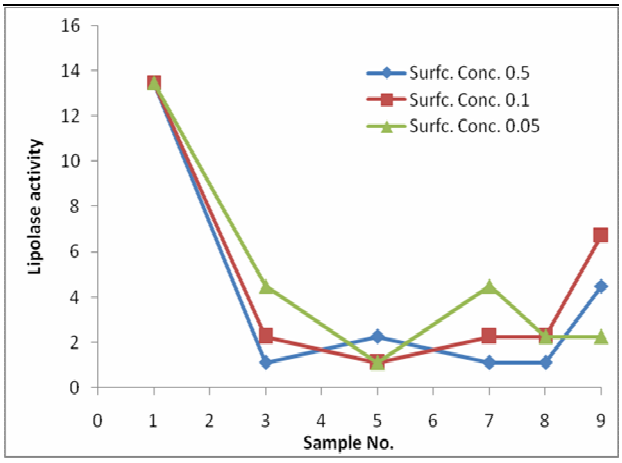


Figure 3.8a: Chart of Lipolase activity at 40°C and different surfactant concentrations

Table 3.8b: Lipolase(100T) activity (Unit/mL) at 50°C

	Temp. 50°C		
Sample No.	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	22.5	22.5	22.5
3	2.25	4.50	6.75
5	4.50	2.25	2.25
7	2.25	4.50	6.75
8	2.25	4.50	4.50
9	6.75	9.00	6.75

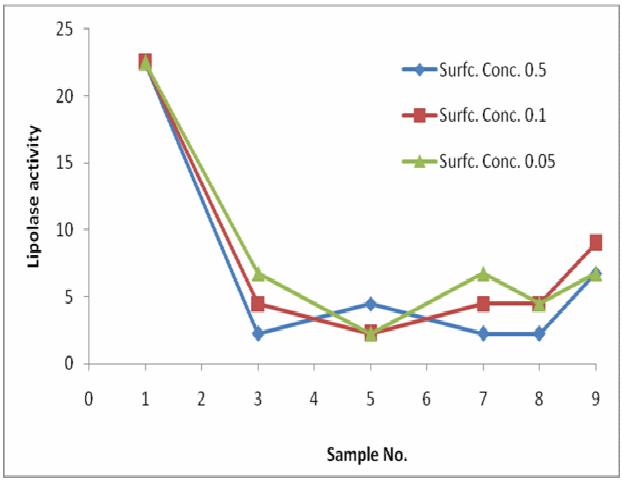


Figure 3.8b: Chart of Lipolase activity at 50°C and different surfactant concentrations

Table 3.8c: Lipolase(100T) activity (Unit/mL) at 60°C

	Temp. 60°C		
Sample No.	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	31.5	31.5	31.5
3	4.50	6.75	11.3
5	9.00	6.75	4.50
7	4.50	6.75	9.00
8	4.50	11.3	6.75
9	9.00	15.8	9.00

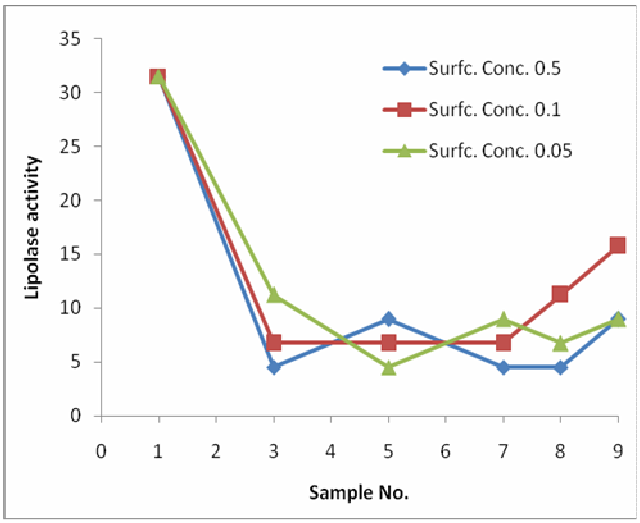


Figure 3.8c: Charts of Lipolase activity at 60°C and different surfactant concentrations

Results analysis have shown in this basic medium at the pH 8.5 that the results are not clear as they are in the normal medium pH 7, it is observed that irregularity in the results of different variables. These results can be summarized as follows:

- Samples which only contain enzyme - sample number one - in all other variables which maintain the top effectiveness of the enzyme, but less values than in normal medium.

- Decreasing in the activity of the enzyme is common in the all other samples compared with their counterparts in the normal medium with the maintenance of the least negative impact on the activity of the samples which contain NP6.

A sharp decline in the activity of the enzyme in the samples that have less concentration, especially in a low temperature. - note Figure 3.7a.

3.2.3. Results analysis at PH 10:

Volumes of NaOH are calculated for **Lipolase 0.1 concentrations** with different surfactant concentrations that is obvious in the given Tables 3.9(a,b,c):

Table 3.9a: Volumes of NaOH at **Lipolase 0.1 concentrations** with different temperature and surfactant concentrations**:**

	surfactant 0.5 conc.			**surfactant 0.1 conc.**			**surfactant 0.05 conc.**		
S No./ T	**40°C**	**50°C**	**60°C**	**40°C**	**50°C**	**60°C**	**40°C**	**50°C**	**60°C**
1	3.0	3.8	4.4	3.0	3.8	4.4	3.0	3.8	4.4
3	0.5	0.4	0.3	0.8	0.6	0.5	1.1	0.8	0.4
5	0.5	0.3	0.2	0.9	0.7	0.4	1.0	1.0	0.9
7	0.2	0.1	0.1	1.0	0.6	0.4	1.1	0.7	0.3
8	0.2	0.1	0.1	0.9	0.5	0.3	0.8	0.6	0.3
9	0.5	0.3	0.2	1.1	0.7	0.4	0.9	0.8	0.5

Table 3.9b: Volumes of NaOH at **Lipolase 0.05 concentrations** with different temperature and surfactant concentrations**:**

	surfactant 0.5 conc.			**surfactant 0.1 conc.**			**surfactant 0.05 conc.**		
S No./ T	**40°C**	**50°C**	**60°C**	**40°C**	**50°C**	**60°C**	**40°C**	**50°C**	**60°C**
1	1.3	1.8	2.4	1.3	1.8	2.4	1.3	1.8	2.4
3	0.3	0.2	0.1	0.4	0.3	0.2	0.5	0.4	0.3
5	0.4	0.4	0.3	0.6	0.5	0.2	0.7	0.6	0.5
7	0.4	0.3	0.2	0.6	0.4	0.3	0.4	0.3	0.3
8	0.3	0.2	0.1	0.4	0.3	0.1	0.5	0.5	0.3
9	0.4	0.2	0.1	0.5	0.4	0.2	0.6	0.5	0.3

Table 3.9c: Volumes of NaOH at **Lipolase 0.01 concentrations** with different temperature and surfactant concentrations**.**

	surfactant 0.5 conc.			**surfactant 0.1 conc.**			**surfactant 0.05 conc.**		
S No./ T	**40°C**	**50°C**	**60°C**	**40°C**	**50°C**	**60°C**	**40°C**	**50°C**	**60°C**
1	0.8	1.2	1.5	0.8	1.2	1.5	0.8	1.2	1.5
3	0.1	0.1	0.05	0.2	0.1	0.1	0.4	0.3	0.2
5	0.3	0.2	0.1	0.5	0.4	0.3	0.7	0.5	0.3
7	0.2	0.1	0.1	0.4	0.3	0.2	0.5	0.4	0.3
8	0.2	0.1	0.05	0.2	0.1	0.1	0.3	0.2	0.1
9	0.3	0.1	0.1	0.4	0.3	0.1	0.5	0.2	0.2

3.2.3.1. Charts for results analysis at PH 10:

Tables of Lipolase(100T) activity (Unit/mL) at different temperatures and surfactant concentrations and their charts:

- First at **Lipolase (100T) 0.1 concentration** and different surfactant concentrations, and this is clear in Table 3.10(a,b,c) and Figure 3.10(a,b,c).

Table 3.10a: Lipolase(100T) activity (Unit/mL) at 40°C

	Temp. 40°C		
Sample No.	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	67.5	67.5	67.5
3	11.3	18.0	24.8
5	11.3	20.3	22.5
7	4.50	22.5	24.8
8	4.50	20.3	18.0
9	11.3	24.8	20.3

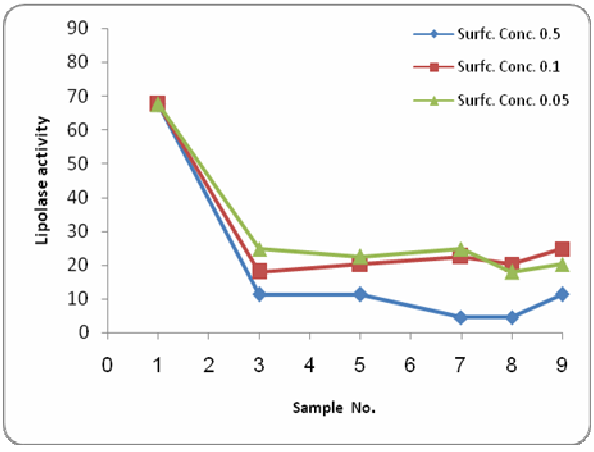


Figure 3.10a: Chart of Lipolase activity at 40°C and different surfactant concentrations .

Table 3.10b: Lipolase(100T) activity (Unit/mL) at 50°C

Sample No.	Temp. 50°C		
	Surf. 0.5	Surf. 0.1	Surf. 0.05
1	85.5	85.5	85.5
3	9.00	13.5	18.0
5	6.80	15.8	22.5
7	2.30	13.5	15.8
8	2.30	11.3	13.5
9	6.80	15.8	18.0

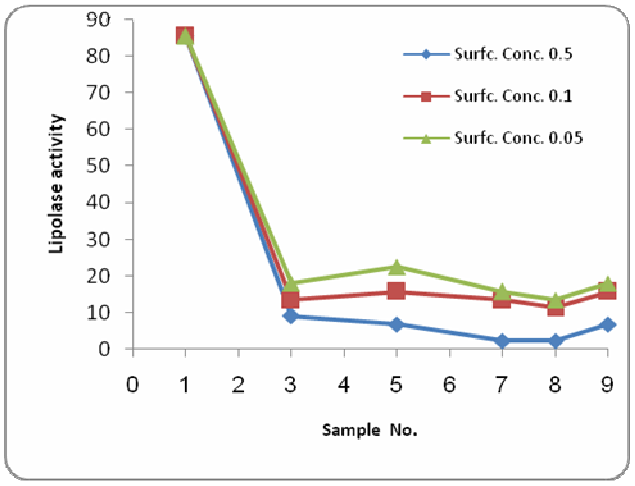


Figure 3.10b: Chart of Lipolase activity at 50°C and different surfactant concentrations .

Table 3.10c: Lipolase(100T) activity (Unit/mL) at 60°C

Sample No.	Temp. 60°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	99.0	99.0	99.0
3	6.80	11.3	13.5
5	4.50	9.00	20.3
7	2.30	9.00	6.80
8	2.30	6.80	6.80
9	4.50	9.00	11.3

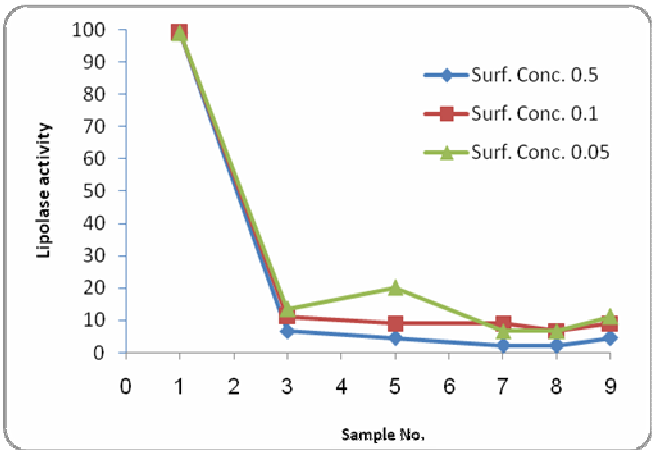


Figure 3.10c: Charts of Lipolase activity at 60°C and different surfactant concentrations

- Second: At **Lipolase (100T) 0.05 concentration** and different surfactant concentrations , and this is clear in Tables 3.11(a,b,c) and Figures 3.11(a,b,c).

Table 3.11a: Lipolase(100T) activity (Unit/mL) at 40°C

	Temp. 40°C		
Sample No.	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	29.3	29.3	29.3
3	6.80	9.00	11.3
5	9.00	13.5	15.8
7	9.00	13.5	9.00
8	6.80	9.00	11.3
9	9.00	11.3	13.5

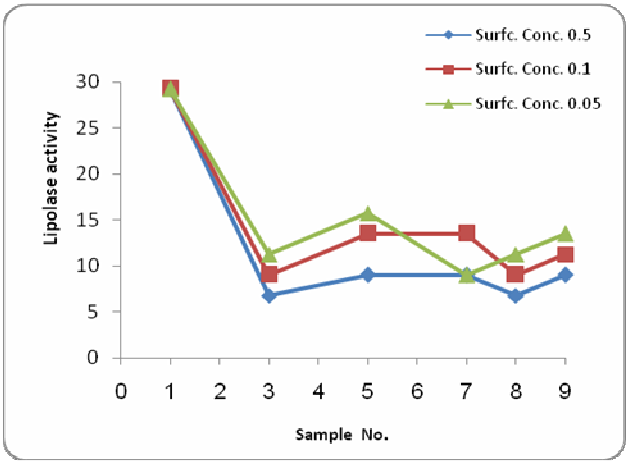


Figure 3.11a: Chart of Lipolase activity at 40°C and different surfactant concentrations

Table 3.11b: Lipolase(100T) activity (Unit/mL) at 50°C

	Temp. 50°C		
Sample No.	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	40.5	40.5	40.5
3	4.50	6.75	9.00
5	9.00	11.3	13.5
7	6.80	9.00	6.75
8	4.50	6.75	11.3
9	4.50	9.00	11.3

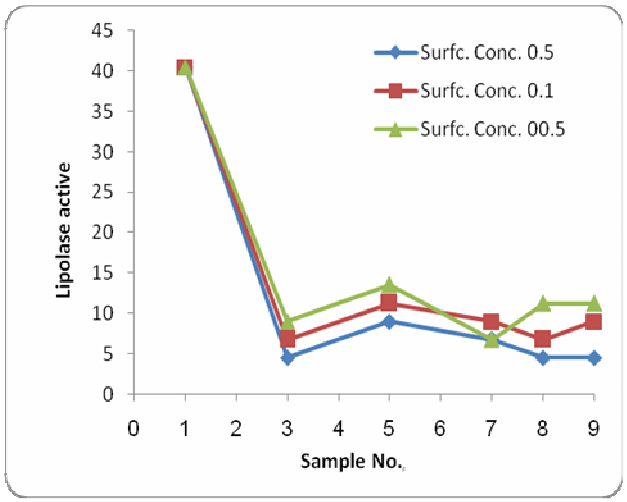


Figure 3.11b: Charts of Lipolase activity at 50°C and different surfactant concentrations

Table 3.11c: Lipolase(100T) activity (Unit/mL) at 60°C

Sample No.	Temp. 60°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	54.0	54.0	54.0
3	2.25	4.50	6.75
5	6.75	4.50	11.3
7	4.50	6.75	6.75
8	2.25	2.25	6.75
9	2.25	4.50	6.75

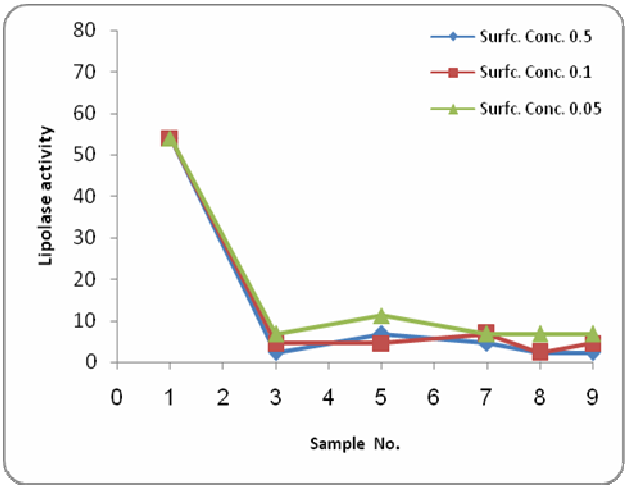


Figure 3.11c: Chart of Lipolase activity at 60°C and different Surfactant concentration

- Third: At **Lipolase (100T) 0.01 concentration** and different surfactant concentrations, and this clear in Tables 3.12(a,b,c) and Figures 3.12(a,b,c).

Table 3.12a: Lipolase(100T) activity (Unit/mL) at 40°C

Sample No.	Temp. 40°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	18.0	18.0	18.0
3	2.25	4.50	9.00
5	6.75	11.25	15.75
7	4.50	9.00	11.25
8	4.50	4.50	6.75
9	6.75	9.00	11.3

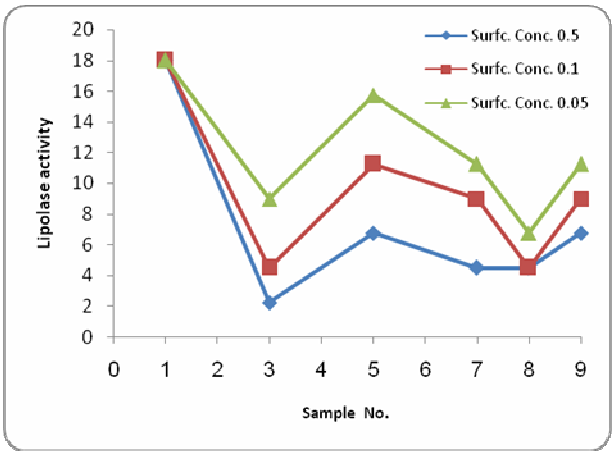


Figure 3.12a: Chart of Lipolase activity at 40°C and different surfactant concentrations

Table 3.12b: Lipolase(100T) activity (Unit/mL) at 50°C

Sample No.	Temp. 50°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	27.0	27.0	27.0
3	2.25	2.25	6.75
5	4.50	9.00	11.3
7	2.25	6.75	9.00
8	2.25	2.25	4.50
9	2.25	6.75	4.50

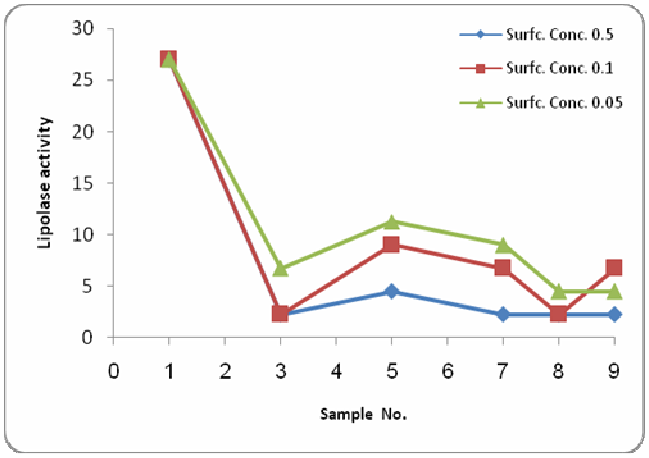


Figure 3.12b: Chart of Lipolase activity at 50°C and different surfactant concentrations

Table 3.12c: Lipolase(100T) activity (Unit/mL) at 60°C

Sample No.	Temp. 60°C		
	Surf. 0.50	Surf. 0.10	Surf. 0.05
1	33.8	33.8	33.8
3	1.13	2.25	4.50
5	2.25	6.75	6.75
7	2.25	4.50	6.75
8	1.13	2.25	2.25
9	2.25	2.25	2.25

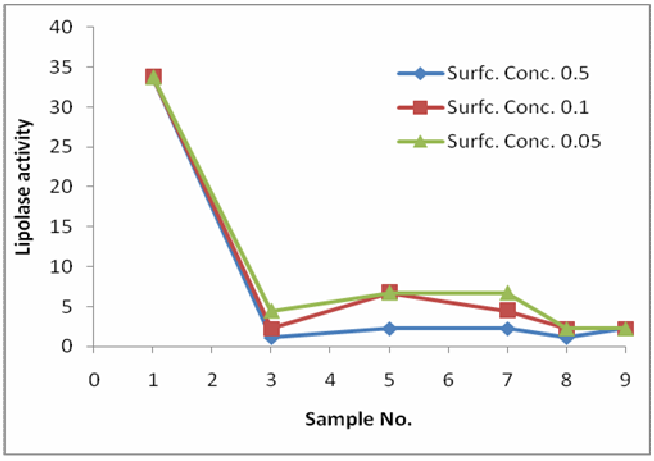


Figure 3.12c: Charts of Lipolase activity at 60°C and different surfactant concentrations

In high basic medium (pH10), continuation of the negative deep impact on the activity of the enzyme, means further decline in effectiveness. Maintaining the optimum activity in the samples without surfactant. Which leads that Lipolase 100T is stable at a wide range of pH.

The samples containing NP6 were observed in this medium that become more negative from the LAS on the activity of the enzyme. This clearly shows the significant negative impact of high basic medium on the nonionic surfactant.

It is clear from the analysis of the first section of this study that the enzyme Lipolase 100T is more effective alone without surfactant and with relatively high temperatures. The study also shows that the LAS inhibits enzymes activity as usual. More importantly, the study shows that NP6 inhibits the enzyme, although the other nonionic surfactant is usually effective on enzymes.

3.3 Analysis of section two

The ten optimum samples that have the best results of detergent solutions were selected after analyzing all the results in section one, see Table 3.13:

Table 3.13: The optimum ten samples that are obtained from the analysis of results.

No.	Sample No.	Relative Activity	Enzyme Conc.	Surfactant Conc.	PH Medium	Temp.
1	1	99.0	0.10	0.00	10.0	60°C
2	1	87.8	0.10	0.00	7.00	60°C
3	1	85.5	0.10	0.00	10.0	50°C
4	1	74.3	0.10	0.00	8.50	60°C
5	1	67.5	0.10	0.00	10.0	40°C
6	1	63.0	0.05	0.00	7.00	60°C
7	1	60.8	0.10	0.00	8.50	50°C
8	1	58.5	0.05	0.00	8.50	60°C
9	5	54.0	0.05	NP6 0.05	8.50	60°C
10	1	54.0	0.05	0.00	7.00	50°C

After preparing the pieces of cloth that are stained with oil-stains. High précised images were taken. These are washed with huge quantities of the selected ten samples in the washing machine. This model of washing machine is made specifically for this purpose. More précised images are taken of the above shown pieces of cloth for comparison, see Figures 13(a,b,c).

3.3.1. Images for pieces of cloth before and after washing:

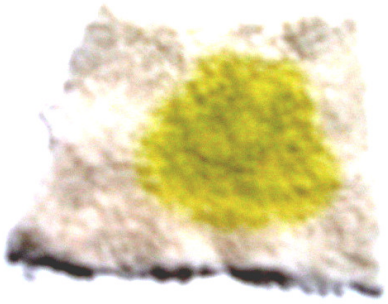


Figure 13a: A piece of cloth that is stained by olive oil stains before it is washed.

Figure 13b : A piece of cloth that is stained by olive oil stains is washed without enzyme.

Figure 13c : A piece of cloth that is stained by olive oil stains is washed with enzyme.

The pictures of previous pieces of washed cloth in different laundry solutions were showed the importance of enzymes in the process of washing. The second picture appeared in some of the remnants of the oil spots because there is no enzyme in this washing solution. Where as the third picture fully showed the removal of the stain.

These cases are applied on all the pieces which are washed in presence and the absence of enzymes with laundry solutions.

After washing many pieces of cloth with the previous washing solutions, differences are observed clearly. Washing shows that enzymatic detergents have a major role in the process of washing .

Part four

Conclusion

Conclusion

What has been reached in this study is a set of facts which confirm the previous work, and examined the new results with regard to the effect of surfactant on Lipolase 100T enzyme.

This study has supported the previous findings, which say that the LAS surfactant works to inhibit the work of the enzyme significantly. Whereas the studies which were conducted on nonionic surfactant show that the activity of the enzyme increases with its presence. It showed that a NP6 - nonionic surfactant - doesn't increase the activity of the enzyme, but it also inhibits the enzyme to less degree than LAS in the two medium normal and basic (pH 8.5), while it becomes more inhibiting than LAS in most of the samples that were studied in the high basic medium (pH 10).

The presence of surfactants in the reaction medium can affect the rate of hydrolysis due to the formation of enzyme–surfactant complexes, the catalytic properties of which may differ from those of the original enzyme in either a positive or a negative manner.

The results were discussed and compared between high and low surfactant concentrations that used in this study.

The samples that did not contain the enzyme in different conditions of surfactant concentration, temperature and pHs values have little impact on the enzyme activity compared with the samples containing the enzyme.

Samples assigned as number 1, which did not contain surfactant are the most effective, because there was no adverse effect on them. The study carried out under different pHs and temperatures showed that the best results are obtained at pH 10 and 60^{o}C.

Samples assigned as number 3, which contained LAS at different pH values and enzyme concentrations showed no change in the enzyme activity by increasing either surfactant concentration or temperature. This leads to optimize the surfactant concentration for purpose

of economy. Study of the samples assigned as number 5, which contained NP6, showed that increasing the temperature and surfactant concentration increasing the enzyme activity.

Study of the samples assigned as number 7 which contained mixture of LAS and NP6 with equal percentages, showed that the enzyme activity is increased at pH 7 and pH 8.5 with increasing temperature at low surfactant concentration in most of the samples, but this changed in pH 10.

Study of samples assigned as number 8 which contained higher percentage of LAS than NP6, showed the same results as samples assigned as number 7, but with more decline in the enzyme activity. Samples assigned as number 9 which contained low percent of LAS than NP6 showed the same results as sample assigned as number 8, but with increased in enzyme activity.

The study also demonstrated that the enzyme Lipolase 100T has a wide range of stability in the basic mediums (7-10) when it is alone without surfactant, while decreasing the stability in the presence of ionic and nonionic surfactant as we had said, but the disparity in favor of nonionic at the presence of surfactant and values of pH in the samples.

It is found that the activity of the enzyme increases with increasing temperature until the extent of not more than about 68^{o}C, but the enzyme breaks over this temperature.

At the opposite of what the LAS does, the NP6 has less inhibiting work in high concentration. Inhibiting is increasing at less concentration in the normal and basic (pH8.5) mediums, while LAS becomes less inhibiting than NP6 in the basic (pH10) medium.

In comparing the pieces of cloth that are washed by the presence of the enzyme. It proves the importance of enzymes in the process of washing.

Table 4.1: The best solutions that could be used reasonably in the process of washing.

No.	Sample No.	Surf.	Relative activity	Enzyme Conc.	Temp.	PH Medium
1	9	0.50	24.8	0.10	40°C	7.00
2	9	0.50	24.8	0.10	60°C	7.00
3	9	0.50	24.8	0.10	60°C	7.00
4	9	0.10	24.8	0.10	40°C	10.0
5	7	0.10	22.5	0.10	40°C	10.0
6	7	0.10	20.3	0.05	60°C	7.00
7	8	0.10	20.3	0.10	40°C	10.0
8	9	0.05	20.3	0.05	40°C	10.0
9	9	0.50	20.0	0.05	50°C	7.00
10	9	0.50	18.0	0.05	40°C	7.00

Solutions in the table above are closer to the logic in the preparation of washing solutions. Because of the economic and psychological reasons for who is carried out the washing. Surfactant Indispensable to its task in the process of washing. To keep the dirt suspended, foams, and other reasonable reasons.

Part four

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تأثير أنزيم الليبيز على لينير ألكيل بنزين سلفونات (LAS) و على نونيل فينول ايثوكسيلات (NP6) في مساحيق الغسيل .

إعداد : لؤي محمد أحمد درويش

المشرف : د . إبراهيم كيالي

ملخص

تعتبر المنظفات من أهم الصناعات الحديثة لما لها أهمية في حياتنا اليومية , ومن أهم هذه المنظفات مساحيق الغسيل وتراكيبها المختلفة وما تحتويه من سرفكتنت " Surfactant " وأنزيمات وغيرها , ومن هذه المواد السرفكتنت الأيوني LAS وغير الأيوني NP6 وأنزيم الليبيز , وهذه الثلاثة موضوع البحث هذا .

إن موضوع البحث في هذه الرسالة هو كيف يمكن أزاله الأوساخ الصعبة وخاصة الدهنية منها عن الملابس باستخدام اقل ما يمكن من تراكيز السرفكتنت والأنزيمات وخفض درجات حرارة الماء المستخدم في التنظيف وذلك بسبب ارتفاع أسعار هذه المواد وتكاليف إنتاجها . واختيار القيم المثلى لهذه المتغيرات التي تساعد في عملية التنظيف .

لقد تم اختيار زيت الزيتون كمصدر للأوساخ الدهنية واستخدم أنزيم الليبوليز T100 لإزالة هذه الأوساخ ضمن تراكيب من مساحيق الغسيل تتضمن LAS و NP6 في ظروف متغيرة من درجات الحرارة وقيم إل pH لمحاليل هذه المواد , وتتم دراسة تأثير هذه المواد على فاعلية الإنزيم عن طريق تحضير محاليل مختلفة التراكيب والتراكيز منها وإجراء عملية تهـ "Hydrolysis" للزيت للحصول على الأحماض الدهنية التي بدورها تقلل من قيم إل pH , ثم تحديد . بعمل معايرة للمحاليل التي ينتمي إليها , و من ثم

حساب فاعلية الإنزيم واختيار الظروف والتراكيب المثلى لمحاليل عملية التنظيف , كذلك تحديد تأثير نوع السرفكتنت وتركيزه على فاعلية الأنزيم .

من جهة أخرى تم غسل قطع من الملابس المختلفة بأفضل النتائج للمحاليل السابقة باستخدام نموذج لغسالة تم صنعها خصيصا لهذه الدراسة , بحيث تم تحديد أهمية الأنزيم في عملية التنظيف بملاحظة الفروق بين قطع الملابس المغسولة وغير المغسولة .

لقد أظهرت الدراسة إن أنزيم الليبوليز T100 له تأثير ايجابي على فاعلية مساحيق التنظيف , وتكون فاعليته كبيرة عندما يكون وحده بدون أي نوع من السرفكتنت وبتركيز مرتفع , بينما تقل فاعليته بشكل كبير بوجود تراكيز عالية من LAS , كذلك تقل فاعليته بوجود NP6 لكن بتأثير اقل من LAS , وهذا غير معهود عن السرفكتنت الغير أيونية التي في مجملها تزيد من فاعلية الأنزيمات .

أيضا تلعب درجة الحرارة دورا مهما في فاعلية الأنزيم , حيث أثبتت الدراسة إن فاعلية الليبوليز T100 تكون عالية عند درجات الحرارة ما بين 50- 60س^0 , كذلك في الوسط المتعادل (pH7) هو الأفضل لفاعلية الأنزيم , بينما تقل الفاعلية بارتفاع قيم pH , في حين إن استقرار الأنزيم كان واضحا عندما يكون وحده دون سرفكتنت في مدى واسع من pH (7 – 10) .