Deanship of Graduate Studies
Al-Quds University

(Evolution in Enhancement Oral Bioavailability of Atorvastatin and Stability of Hydroquinone Topical Gel) Based on Microemulsion Phase Behavior of Tri-Block Co-polymer: Poloxamer 188

Mayada Nehad Mustafa

M.Sc. Thesis

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Thesis Approval

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1- Head of Committee: Prof. Ibrahim Kayali Signature …….
2- Internal Examiner: Signature ……..….
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Jerusalem-Palestine

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Dedication:

I would like to dedicate this work to my father and my family, thank you is not enough to express the deep amazing feeling you gave to me during the journey of this research. To you Hani for your endless support and encouragement. To my friends recognition of their efforts for encourage me to complete this master thesis.
To Palestine.
Thank you all …

Mayada Nehad Mustafa Mustafa

Declaration:

I Certify that this thesis submitted for the degree of Master is my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed:

(Mayada Nehad Mustafa Mustafa)

Date: / / 2013

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Abstract

Phase behavior of Tri-block copolymer (polyethylene oxide)$_a$(polypropylene oxide)$_b$(Polyethylene oxide)$_a$, poloxamer 188, and microstructure were studied in aternary system of non polar Lime oil and water, the effect of temperature and addition of co-solvent and co-surfactant on tri-block copolymer behavior was also investigated in separate systems. The phase diagram of each system accomplished by drop wise titration method and constructed depending on the obtained results a five phase diagram of poloxamer 188, pharmaceutically acceptable propylene glycol as a co-solvent and Tween 80 and cremophor RH40 as a co-surfactant were obtained.

The microstructure phases obtained from microemulsion systems characterized by visual inspection as well as cross polarizer were used to detect anisotropic properties. The clearly identified main phases obtained were gel, cubic, inverse micelles and normal micelles with clearly appearing the effect of addition of co-solvent and co-surfactant on the obtained phases on each system. The temperature clearly affects the behavior of poloxamer 188 with presence of co-solvent and co-surfactant by increasing the solubility in oil rich region of ternary phase diagram with increasing temperature.

Enhance bioavailability of poorly water soluble Atorvastatin Calcium by constructing a microemulsion formulation depending on the behavior of poloxamer with biocompatible solvent and surfactant. The stability of Atorvatstain formulation tested for microemulsion properties and evaluated for clarity, precipitation, viscosity determination, and drug content by constructing analytical test method based on High Performance Liquid Chromatography (HPLC). The prepared formulations investigated through stability program including ambient and stress conditions of temperature and relative humidity for three months.

Microemulsion as a topical drug delivery system for Hydroquinone prepared depending on the obtained results of microemulsion systems including poloxamer 188 as main surfactant. Each component in the formulation was properly selected for serving for both an effective component in microemulson formulation and achieving cosmetics objectives as skin compatibility, smell and perfuming characteristics, effective penetration enhancer and achieving customer compliance, beside ease of preparation and earning satisfaction for industrial point of view. The prepared formulations of Hydroquinone topical dosage form was investigated for clarity, precipitation, pH tests and viscosity determination. The stability results of Hydroquinone topical formulation obtained by investigating the formulation through stability program including ambient and stress conditions of temperatures and relative humidity with compliance of Palestinian Ministry of Health guidelines. The behavior and microstructure of tri-block copolymer can be involved in stable, compatible and safe products for pharmaceutical and cosmetics applications.

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List of Abbreviations

No.	Abbreviations	Read as
1.	API	Active Pharmaceutical Ingredient
2.	BE	Bioavailability
3.	BHT	Butylated hydroxytoluene
4.	BP	British Pharmacopoeia
5.	CMC	Critical micelle concentration
6.	CMT	Critical Micellization Temperature
7.	EOR	Enhanced oil recovery
8.	Eur. P.	European Pharmacopoeia
9.	HPLC	High Performance Liquid Chromatography
10.	IFT	Inter facial tension
11.	NMT	Not More Than
12.	PPO	Poly Propylene oxide
13.	PEO	Poly Ethylene oxide
14.	RH	Relative humidity
15.	RT	Retention time
16.	SEAR	Surfactants enhanced aquifer remediation
17.	SMEDDS	Self Microemulsifing drug delivery system
18.	UN	Undetected
19.	USP-34	United State Pharmacopeia
20.	WOR	Water: Oil ratio
21.	INCI	International Nomenclature of Cosmetic Ingredients
22.	SAXS	Small Angle X-ray Scattering

Chapter One
Introduction

Chapter one
Introduction

1.1 Microemulsion

Microemulsions are a thermodynamic stable isotropically clear dispersion of tow immiscible liquids such as oil and water stabilized by the interfacial film of surfactants with a combination of additives or co-surfactant. Microemulsions in comparison to other unstable dispersions such as emulsions or suspensions show advantages in terms of better solubilization potential and thermodynamic stability, since they are prepared by little energy input (heat and mixing) and have a long shelf life. The term microemulsion was first coined by Schulman group and still a rich scientific regain for research and magnificent finding till today.(Satya Priya and Animesh Kumar, 2006).

Some of the unique factors related to microemulsion are the presence of different texture such as oil droplets in water, water droplets in oil, bi-continuous, lamellar mixtures which are formed by their particular ability to significantly reduce interfacial tension (IFT) and enhance the solubilization of hydrophilic and lipophilic ingredients with aid of different factors such as electrolytes and temperature, in order to indicate the existence of these regions a phase study is constructed depending on the composition ratios.
Construction of phase diagram can determine the aqueous dilutability and the range of composition of monophasic regions, the different structure formed by Microemulsions are typically classified into three main categories, or Winsor-type systems. (Lee, 2010).

Winsor's type I microemulsion consist of oil swollen micelles in water with excess water, where as a type II system consists of water swollen inverse micelles in oil with excess oil. Type III microemulsion system, middle phase microemulsion or Winsor III appears with an excess of both water and oil this type can be understood as an accumulation of all swollen micelles so they touch one another, forming some kind of percolated dispersion or a perfectly bi-continuous structure in which all water domains are connected and all oil domains are likewise connected. (Salager, et al. 2005).

Since microemulsion are not dispersions, the boundary between water and oil domains do not exhibit a strong curvature and the best microemulsion are formed with a zero-net curvature condition of the surfactant layer between the oil and water domains.
Microemulsion which typically found in the center of a surfactant – oil – water (SOW) ternary phase diagram of Winsor type III which have a unique physicochemical formulation in which the affinity of surfactant for the oil phase exactly matches its affinity to water phase. This situation described by Winsor 50 years ago and has been referred to as " optimum formulation".
To quantify the physicochemical formulation of SOW System many attempts have been made including phase inversion temperature, Hydrophilic – Lipophilic Balance (HLB), cohesive energy ratio and interaction energy ratio R, which was introduced by Winsor more than 50 years ago. (Salager, et al. 2005).
To have a clear understanding of the ideas about Winsor work and the phase diagram as guideline map outlines the most important concept in the microemulsion phase and the roll of the surfactant in microemulsion systems, a simple description can be given to a ternary phase diagram system.

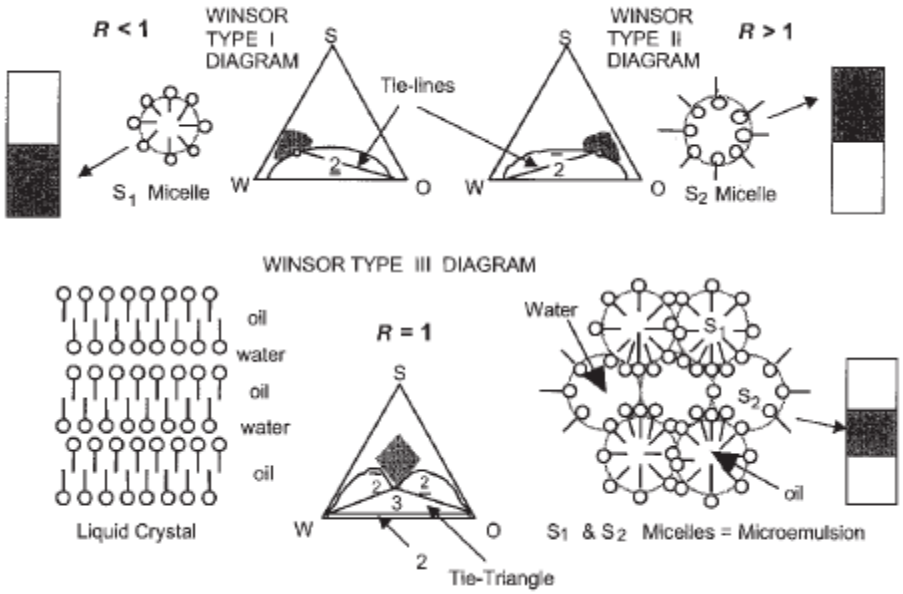

Figure 1. 1: Three types of phase behavior for surfactant (S), oil (O) and water (W) system according to Winsor (adapted from Salager, et al. January, 2005).

Figure (1.1) Illustrate the three types of phase behavior for surfactant (S) – oil (O) – water (W) system according to Winsor. Shading areas indicate the surfactant rich phase, and R is Winsor ratio, that indicates the ratio of interaction between surfactants at interface and oil, and the interaction between surfactants and water. R= A_{co}/A_{cw} . (Minana-Perez, 1995).

In ternary phase diagram each of the vertices corresponds to each pure component : surfactant, oil and water, the composition of the particular mixture of the three component can be given as appoint inside the triangle. In case of Type I and Type II microemulsion system , figure (1.1) describes this simple clarification:
The dome in the lower part of the triangle represent two phases - polyphasic- region and a SOW mixture inside the dome will separate in to two phases as indicated by the Tie-Line.

In Type I microemulsion the separation occurs in the side that produced excess oil in all the side of rich surfactant- rich aqueous phase and the composition of each phase can be determine by locating where the tie line intersects with the dome frontier it is called binodal curve. whereas the intersection of Binodal curve and tie line closest to the water vertex is the surfactant rich water phase, where as the other intersection point in the other side is the oil excess. Conversely in a Winsor Type II two type phase region, the tie line slope is in the opposite direction and the surfactant rich oil is in equilibrium with excess aqueous phase.
A Winsor Type III called middle phase microemulsion because it is located in intermediate in the triangle and contains the tie triangle and any point in the triangle will separate in to three phases, and the composition of the phases is independent where the plot of the tie triangle it's proportional to the component of each corner of the triangle. (Salager, et al. 2005).

Winsor introduced the ratio of interaction (R) between the surfactant, oil and water that form different phases in the diagram, by a relation described as:

$$R = A_{co}/A_{cw}$$

where : A_{co} : Indicates the interaction between surfactant adsorbed on the interface and the oil phase per unit area of interface, and A_{cw} is the same for water phase. by this definition R equals 1 when middle phase microemulsion contains same volume of oil and water. When changes have been introduce to the system such as chemical changes which can be described as change the nature of one of the three components or a physical change such as change in temperature, salinity or pressure at least one of the interactions have to be changed , for example if the salinity in the aqueous phase in increases, by increasing the electrolyte concentration in water, the interaction of A_{cw} is decreased and R will increased, resulting to increase uptake of oil in to the middle phase. (Salager, et al. January, 2005).

When R=1 a maximum solubilization and a very low minimum interfacial tension are occurs.
Figure (1.2) Illustrate phase behaviors along salinity scan test tube aspect and phase diagrams.

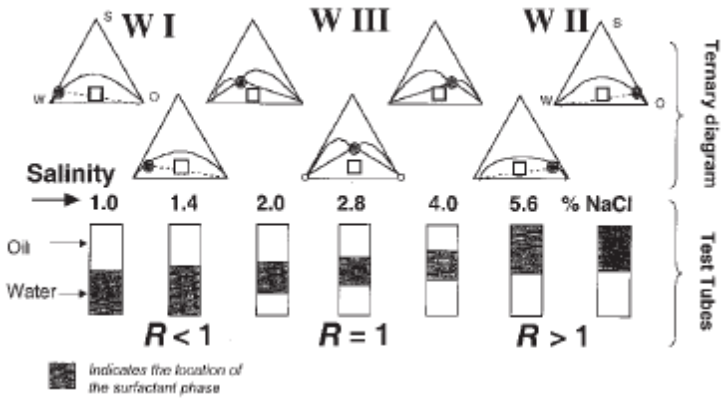

Figure 1.2: Phase behavior along a salinity scan test tube aspect and phase diagrams. (Adapted from Salager, et al. January, 2005).

The figure describe a study on changing just one variable which is salinity, this is called the formulation scan , and it is mostly used when changing one variable of the whole system, since this formulation scan can experimentally describe the best and special situation of having the interfacial tension goes to a very low minimum and solubilization reaches maximum when R= 1, the situation in which the formula take equal uptake of oil and water is known as the optimum formulation, this term is first designed by enhanced oil recovery (EOR) researchers but as work have to be applied in field the theoretical ideas have to approve its reality or some changes have to be introduced, as known in even for relatively simple systems there are much variables have to be considered so a correlation variables have to be introduced, these correlation are related to the free energy of transfer of a surfactant molecule from water to the oil phase (Δ Gw→o) this is also called surfactant affinity difference (SAD). (Kanicky, 2001).

Surfactant affinity difference (SAD):
Ultra-low interfacial tension and maximum oil solubilization can be predicted using numerical empirical correlation.

$$SAD = \mu w - \mu o = \Delta G (w \rightarrow o) = -RTLnKp$$

Where μw and μo are the chemical potential of the surfactant in the water and oil phase, respectively.
R in this case corresponds to the ideal gas constant.
T is the temperature.
Kp is the partition coefficient of surfactant between water and oil.

This correlation (Surfactant affinity difference) is the measure of the difference between the Gibbs free energy of surfactant in the oil and water phase. SAD actually represents the free energy of transfer of a surfactant molecule from the water to the oil.

Many factors can change the hydrophobic balance of the surfactants may enable movement from one type to another. These factors are illustrated below:

1) Chain length of surfactants
   Longer chain length would transfer from Winsor I to Winsor II.
2) Polarity of aqueous phase (Water)
   The addition of short chain alcohol can causes increase in polarity of water and then transfer from Winsor I to Winsor II.
3) Temperature and electrolyte concentration
   The increasing in temperature for a non-ionic surfactants or increasing in electrolyte concentration for ionic surfactants would shift from Winsor I to Winsor II also. (Goodwin, 2004).

The surfactant chosen must be able to lower the Interfacial Tension (IFT) to a very small values which facilitate the dispersion process during microemulsion preparation and provide a flexible film that can readily deform around the droplet and be of a appropriate lipophilic character to provide the correct curvature at the Interfacial regions.
Many numerical equations can be used to calculate the values of interfacial tension (IFT), scientist Huh (1979) develop an equation that used to estimate IFT using optimal solubility, this equation is given as:

$$\gamma = 0.3 / (\sigma^*)^2$$

where γ is IFT and σ* is oil or water solubilization ratio at optimal salinity. (Flaaten, 2007).

Another research indicates that interfacial tension can be calculated using Vannegut equation (1942):

$$\gamma = \pi (\rho_1 - \rho_2) R_d^3 \omega^2 / 4$$

γ = Interfacial tension
ρ_1= Density of continues phase
ρ_2= Density of droplet phase

R_d = Droplet diameter
ω = Rotational speed. (Flaaten, 2007).

All the above results of scientific research where obtained to ease the dealing of IFT values in equation forms, since these values are of great importance in microemulsion techniques.

Microemulsion as a technique has increasing interest in all scientific approaches, mixing of water, oil and surfactant or mixture of surfactants, to get special formulation proper for certain branch in industry, cosmetics and pharmacy products is the riches area for research. The most important properties of microemulsion such as ultralow interfacial tension, small micelles size, transparency, are the most driving forces for searching for good, stable and multipurpose microemulsion formulas.

Preparation of microemulsion to ease the solubility of non polar oils in such formulation and get a maximum solubilization is the global idea of microemulsion application, and the logical ways to enhance the solubilization is the increase in interaction of the surfactant for both the oil and water phase, which can be achieved by carefully increasing the volume for both the head and the tail of the surfactant molecule, or addition of molecules that bridge the adsorbent surfactant layer and the bulk phase, and this is accomplished by using lipophilic and hydrophilic linker effect, this linker result in what is called extended surfactant, or using block copolymer additives, this is what is the most interesting idea in using a type of polymers a tri-block co-polymer known by many names such as poloxamer, Pluronics, synbyronic, or Lutrol.

polymers have a great attention in recent years as a main component in microemulsion formulation as main surfactant or as co-surfactant , the Surfactant polymer interaction have been long studied in various application ranging from detergency to enhance oil recovery, to drug and gene delivery. Many different factors affecting the interaction. The type of surfactant (ionic, nonionic, cationic), surfactant chain length, the ionic strength of aqueous phase. this interaction activate each part of the new complex to be more effective than the parent component. (Monica, et al. 2008).

Microemulsion has emerged a prospective approach in cosmetics industry, since the formulation can incorporate a large amount of lipophilic cosmetics actives in to the inner oil phase. Due to the small droplet size and large amount of inner phase, the density of droplets and their surface area are assumed to be high, therefore the droplet settle down to close contact with skin providing high concentration gradient and improve permeability , moreover low surface tension ensure good contact to the skin. The surfactant and co-surfactant in the microemulsion can reduce the diffusion barrier of the stratum correnum by acting penetration enhancers and facilitating cutaneous penetration. (Adnan, et al. 2008)

1.2 Characterization of Tri-block copolymer" Poloxamer"

Polymers are substances of high molecular weight made up of repeating building units known as monomers, used in pharmaceuticals applications as suspending agent,

emulsifying agent, adhesives, coating materials, a components in controlled release dosage forms, and in site specific or targets dosage forms as a drug delivery system.

The chemical reactivity of the polymers determined by its monomer units chemical reactivity, polymers could be linear or branched in their molecular shape, polymers in which all their monomers are identical are identified as homo-polymers, and those formed from more than one monomer type are identified as copolymers. Copolymers may be described as alternating copolymers, block copolymers, or graft copolymers. Pluronic is one of the most widely used block copolymers. (Florence, 1998).

Tri- block copolymer known for BASF chemical corporation are used under name of pluronic or poloxamer are available as di-functional block copolymers of non-ionic nature comprises from a central block of relatively hydrophobic polypropylene oxide surrounding on both sides by the blocks of relatively hydrophilic polyethylene oxide.(Jakobs, et al. 1999). Due to this unique structure poloxamers can form different micelles structures when immersed in different solvents, especially when the solvent is selective for the building block of the polymer. (Alexandridis, et al. 1995).

The polymerization process define the molecular weight distribution of the polymer, during the polymerization process of propylene oxide block a fraction of propylene oxide may change to allyl alcohol which farther will react with propylene oxide and ethylene oxide forming polymeric material, with half molecular weight of the final wanted product, this segment leads to unsaturation part of the poloxamer. Finally the reaction terminated by hydroxyl group so no farther reactions will occur and polymerization process stopped. (Guiader, 2005).

The following figure describe polymerization process of the poloxamer:

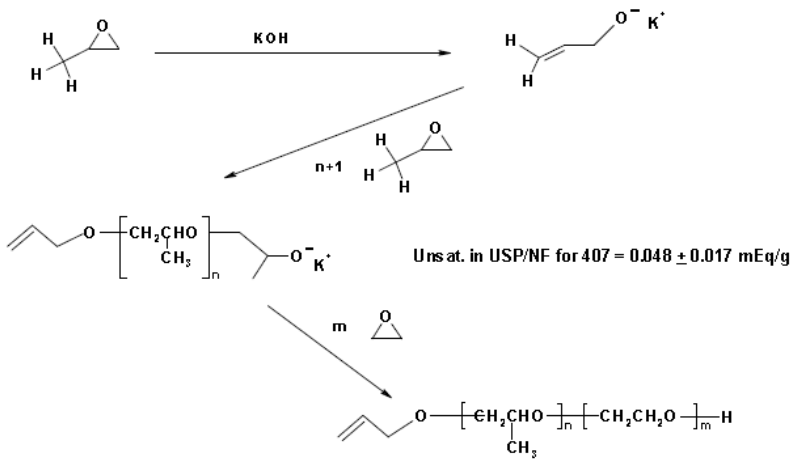

Figure 1.3: Description of the polymerization process of Poloxamer (Guiader, 2005).

poloxamers have the structure: $HO(C_2H_4O)a(C_3H_6O)b(C_2H_4O)aH$, where a and b are block with following values (Hand Book of Pharmaceutical Excipients, 2009):

Table (1.1): Types of Tri-block Copolymer.

Pluronic	**Poloxamer**	**a**	**b**	**Ave molecular weight**
F127NF	407	101	56	9 840–14 600
F108NF	338	141	44	12 700–17 400
F87NF	237	64	37	6 840–8 830
F68NF	188	80	27	7 680–9 510
L44NF	124	12	20	2 090–2 360

These Tri-block copolymers listed above have specifications which are conformed to the monograph of the official requirements for different international references such as United Sates Pharmacopoeia, British Pharmacopeia, European Pharmacopeia, and Hand Book of Pharmaceutical Excipients, even there is five grades of poloxamer are listed on United state pharmacopeia, there is other commercial grades of poloxamer. (Hand Book of Pharmaceutical Excipients, 2009)

Table (1.2): Specification of Tri-block copolymer.

Specification	**Tri-block copolymer**				
	124	188	237	338	407
Physical Form	Liquid	Solid	Solid	Solid	Solid
pH, 2.5% aqueous	5.0 – 7.5	5.0 – 7.5	5.0 – 7.5	5.0 – 7.5	5.0 – 7.5
Cloud point, 10%	71–75C	>100 C	>100 C	>100 C	>100 C
H_2O%	0.4 max.	0.4 max.	0.4 max.	0.4 max.	0.4 max.
BHT, ppm	–	50– 125	50– 125	50– 125	50– 125
Weight % oxyethylene	46.7 ± 1.9	81.8 ± 1.9	72.4 ± 1.9	83.1 ± 1.7	73.2 ± 1.7
Ethylene Oxide, ppm	1 max.	1 max.	1 max.	1 max.	1 max.
Propylene Oxide, ppm	5 max	5 max	5 max	5 max	5 max
1,4 dioxane, ppm	5 max	5 max	5 max	5 max	5 max
Heavy Metals, ppm	2 max.	2 max.	2 max.	2 max.	2 max.
Melting point	168C	52–578C	498C	578C	52–578C
Density at 258C (g/cm3)	1.06	1.06	1.06	1.06	1.06
Flash point	260C	260C	260C	260C	260C
Solubility in water and ethanol (95%)	Freely Soluble	Freely Soluble	Freely Soluble	Freely Soluble	Freely Soluble
HLB Value	12 - 18	> 24	> 24	> 24	18 - 23

The chemical structure of pluronics described on the following figure:

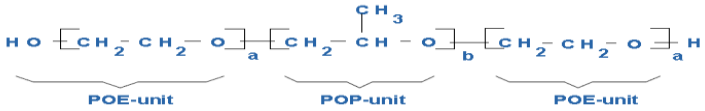

Figure 1. 4: Chemical structure of pluronic (adapted from reference (Guiader, 2005).

Poloxamer can be available in micronized particle size, BASF Corporation are the leading company in worldwide marketing of all grades of tri-block copolymers have developed a new type of tri-block copolymers which are Lutrol micro 68 and Lutrol micro 127 to enhance the solubility of poorly water soluble drug.

of special grades of Lutrol F68 known as microprilled Lutrol F68 with much effective properties related to Average particle size of 50 micron, Stronger solubilization activities, Controlled dissolution rate, Achievement of homogeneous blend, Elimination of dose dumping, Effective water soluble lubricant all of these newly attractive properties are developed to ease the use of tri-block copolymer in all application. Some application of Lutrol F68 in pharmaceutical applications Lutrol F 68 is primarily applied as an emulsifier, solubilizers, and suspension stabilizer in liquid oral, topical and parenteral dosage forms. In solid preparations it acts as wetting agent, plasticizer, and to enhance the solubility and bioavailability of sparingly water soluble active drugs. And due to its low toxicity, it is proved to be a very useful excipient, even for parenterally applications. Also the unpleasant taste or smell of actives pharmaceutical materials can be masked by either using polymeric film formers or Lutrol F 68 alone or by combining it with hydrophobic compounds. (Guiader, 2005).

All types of tri-block copolymers have been introduced to many different projects and many research papers have been published including their behavior and the influence of addition solvent, co-surfactant and temperature on their behavior.

1.3 Lime oil:

Lime oil and Lemon oil are natural oils used as flavoring and fragrance raw material in many products, the flower and the fruits of lime could be used to extract the juice. Whereas the fruits and the trees of lime is different from lemon oil, it is first known in Asia, known on International Nomenclature of Cosmetic Ingredients (INCI) as Citrus Aurantifolia and has CAS#: 8008-26-2, (International Cosmetic Ingredient Dictionary and Handbook 2000, 1999).the specification of Lime oil is listed in the table below as acoording to(Sciencelab, 2011).

Table (1.3): Specification of Lime oil.

Specification	Limit
Color	Colorless to Greenish yellow
Odor	Intense fresh citrus
Solubility	Insoluble in water and soluble in organic solvent as ethanol and in propylene glycol
specific gravity of oil	Between 0.855 to 0.863
optical rotation	+34 to +47
Storage Condition	Sensitive to both air and light so the oil has to be tightly kept and in amber bottles

The extracted lime used in different products in food , drinks, Herbal, the Lime flower is listed by the Council of Europe as a natural source of food flavoring (category number 2, which indicate the product can be added to food stuff in small quantities, and categorized as safe product. In herbal uses the lime flower extract are uncountable as in arteriosclerotic hypertension, feverish colds, and specifically for raised arterial pressure associated with arteriosclerosis and nervous tension. It has reported to possess a restricted range of antifungal activity. (Gurin and Reveiller, 1984).

Several processes are used to produce lime oil. Expressed lime oils are produced by pressing the ripe fruits by sponge-press (by hand) or machines. These oils may be produced more economically using an integrated juice-oil procedure such as the Brown Oil Extractor, where citrus fruit is partially submerged in water and abraded by metal discs. The oil is separated from the juice as a water-emulsion, and further separated using centrifugation. (Swaine, 1988).

Lemon and Lime oils are also produced by distillation of expressed oils or direct distillation of fruit. oils are distilled for removal of terpenes in order to improve solubility and permit use for flavoring carbonated beverages, the oils can be steam distilled to remove nonvolatile furocoumarins and marketed as " psoralen free" .(Burdock,1995).

The photo toxicity of lemon and lime oil has been clarified and investigated using samples obtained from different geographic locations, studies have investigate the phototoxicity of a large number of fragrance raw materials including lemon oil and lime oil. Fragrance raw materials were tested using humans, pigs and albino, hairless mice. Several irradiation sources were used including sunlight, a solar simulator and a UVA radiation. Expressed lime oil was phototoxic in all three species under all three radiation sources. But Distilled

lime oil presented no phototoxic response in any species, which is a great results indicate the safety of using the oil by identifying the type of extraction. (Forbes, et al.1977).

1.4 Atorvastatin Calcium:

Atorvastatin is a member of drugs known as statins used for lowering blood cholesterol, it is an inhibitor of 3-hydroxy-3-methylglytarylcoenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate limiting step in cholesterol biosynthesis. Atorvastatin calcium is salt form of the atorvatstain active pharmaceutical ingredient, is a white to off white crystalline powder that is insoluble in aqueous solution of pH 4 and below, very slightly soluble in distilled water, pH 7.4 phosphate buffer, and acetonitrile, slightly soluble in ethanol and freely soluble in methanol.

Atrovastatin calcium is [R-(R*, R*)]-2-(4-fluorophenyl)-(beta),[dgr]-dihydroxy-5-(1-methyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. The empirical formula of atorvastatin calcium is $(C_{33}H_{34}FN_2O_5)_2Ca.3H_2O$ and its molecular weight is 1209.42 its structural formula is:

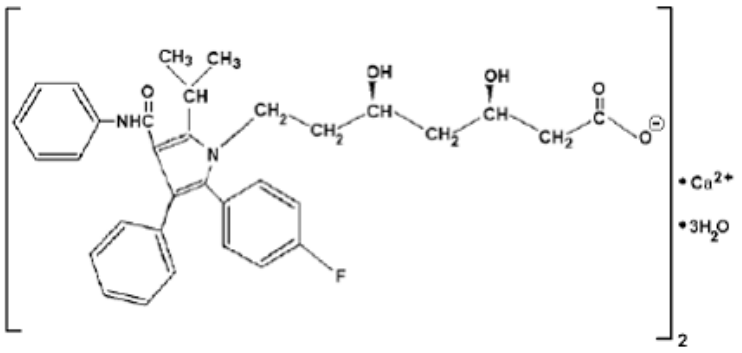

Figure 1.5: chemical structure of atorvastatin calcium (adapted from PDR, 2006).

The Intestinal permeability of Atorvastatin is high as at the physiological intestinal pH ranging (6.0 – 6.5). It is reported that the absolute bioavalability of Atorvastatin is about 12% after a 40mg of oral dosage form. from the chemical structure of Atorvastatin the pKa of Atorvastatin's terminal carboxylic group is 4.5, theoretically , the solubility and dissolution rate of Atorvstatin calcium is markedly improved at pH values equal to or greater than(pKa + 1). The pH of the gastric environment equal or greater than 5.5, so an enhancement in the dissolution and bioavailability can be suggested through formulation with pH ranging more than 5.5. (Salam and Dumitru, 2009).

The oral bioavailability of atorvatstain is limited by many conditions such as the membrane permeability, the solubility, and the dissolution rate which treated as a critical factors for a sparingly water soluble drug. Many approaches have been developed to improve the

solubility of and enhance the dissolution rate and oral bioavailability of poorly soluble Atrovastatin like, salt formation, Solid dispersion, including complex agents, microemulsion, micronization, and self emulsifing drug delivery system.
Physical modification aims to increase the surface area, solubility and wettability of the powder particles and therefore focusing on particle size reduction or generation of amorphous states of the active pharmaceutical ingredients.

Atrovastatin is usually used in its calcium salt as a conventional active in solid dosage forms. Also it can exist in an amorphous form and in many crystalline forms, the main difference between these forms are the dissolution characteristics and bioavailability of the drugs. to have uniform from an amorphous form only of the powder of atorvastatin additional process parameters have to be added which resulting in 5 – 10 % loss in the final amount of atorvastatin calcium, which reduces the economically production process since atorvastatin calcium is an extremely expensive substance. (Salam and Dumitru, 2009).

Atorvastatin calcium is presented as solid tablet dosage form for oral administration, present in strength of 10, 20, 40 and 80 mg per tablet, marketed under trade name Lipitor by Pfizer.(PDR, 2006).

1.5 Hydroquinone:

Hydroquinone is 1,4-benzenediol. It CAS number is [123-31-9]. Hydroquinone is structurally related to monobenzone. Hydroquinone occurs as fine, white needles. It is freely soluble in water and in alcohol with a pKa of 9.96. Chemically, hydroquinone is designated as p-dihydroxybenzene; the empirical formula is $C_6H_6O_2$; molecular weight 110.1.The structural formula is:

Figure 1.6: Hydroquinone Structural Formula [adapted from USP 34]

The USP- 34 assign the limit of melting range of hydroquinone as raw material is between 172 – 174C, the limit of water content: is not more than 0.5% and the limit of residue on ignition is not more than 0.5 %. The critical point in dealing with hydroquinone is its photo stability it has to be stored in a well closed light resistance containers. (USP-34)

Hydroquinone used in many products due to its properties as a water-soluble reducing agent. It is used in medicine (up to 5% in concentration) to treat dyschromias (as melasma, an acquired hypermelanosis) and in cosmetics (up to 2%) as a depigmenting agent in a number of topical skin creams. It may present as gel, cream or solution for topical purposes. (U.S. Food & Drug Administration, 2009).

The main use of hydroquinone as bleaching and defragmentation product has given it a worldwide good presentation and has a high customer satisfaction values since it has a great effect within the usage period. The main side effect of the products containing

hydroquinone that they may cause irritation and as a drug the usage period have to be controlled and not exceeding the prescription dose. (Saraf, et al, 2012).

Different approaches contributed in preparation hydroquinone for topical use, the most known techniques are microemulsion, micro-sponge, liposome. The most interesting properties of using microemulsion over other techniques are:

The ease of preparation, long stability, higher ability of drug loading, can be applied for polar and non polar actives, can be applied in diverse dosage form as creams, lotions and pastes, can maintain compatibility and preventing irritation to sensitive skin, compatible to divers vehicle and component and achieving customer satisfaction. (Saraf, et al, 2012).

Chapter Two
Literature Review

Chapter two
Literature review

Tri-block copolymer of polyethylene oxide and polypropylene oxide often denoted by PEO-PPO-PEO are water soluble and commercially available as nonionic macromolecule surfactant, these tri-block copolymers commercially marketed worldwide because it meets the requirements and achieve the optimum specification and required properties related to variation of molecular characteristic depended on PPO\PEO ratio.
Tri-block copolymers are an important class of surfactant and find widespread industrial application in detergency, dispersion stabilization, foaming, emulsification, lubrication, and in more specialized application in pharmaceutics such as: drug solubilization and controlled release, in bio-processing, as a delivery system and as a drug targeting system. There are interesting research targeting tri-block copolymers this chapter will summarize some of these great research.

Tri-block copolymers like any other surfactants, when dispersed in solution or liquids at low concentrations it exist individually, forms monomolecular micelles, this leads to decrease in the surface tension, as the concentration increases the polymer forms multi-molecular aggregates. The structural properties of the tri-block copolymer offers unique properties, the propylene oxide forms hydrophobic cores where methyl groups interacts by Van der waals forces with medium going solubilization. Water solubility of tri-block copolymers due to hydrogen bonding of polyethylene oxide (PEO) block with water, interaction of ether oxygen with water, these interactions strength tri-block copolymers to become soluble in both polar and non-polar solvents. The aggregation forms of tri-block copolymers depend on many properties as the molecular weight of these polymers, the size of the blocks, the temperature and the solvent components.(BASF, 2010).

Average Molecular weight of tri-block copolymer, determined by the molecular weight of PPO and PEO blocks, affects the main concept of polymer behavior as critical micelles concentration (CMC) and critical micellization temperature (CMT). (BASF, 2010).

(Alexandridids, et al. 1994) published a study on the Critical Micellization temperature (CMT) and Critical Micellization Concentration (CMC) of 12 types of tri-block copolymer from thermodynamic point of view. It was determined that the micellization process is entropy driven and has an endothermic micellization enthalpy. It was found that the hydrophopic part of co-polymer (PPO), was responsible for the micellization, due to dehydration of hydrogen bonding between water and PPO with increasing temperature. The Critical Micellization Concentration (CMC) dependence on temperature and size of head group (PEO) of copolymer follow the same trend as with lower molecular weight of nonionic surfactant were as the effect of temperature a appeared more pronounced with copolymer.

In another published work for (Alexandridis, et al, 1995) Confirmed the effect of temperature increasing on micellization behavior in aqueous solution of copolymer as temperature increases, the proportion of dehydrated methyl groups of polymer increases.

This indicates that methyl groups are experiencing less polar environment and the interaction of methyl groups with water molecules is weakened by heat.

(Wanaka, et al. 1999). Studied the phase diagrams of different types of copolymers and confirmed that a liquid micellar phase is stable at low temperatures but transforms into the cubic structure by increasing the temperature usually there is a broad temperature region of about 20C in which the transformation occurs. At higher concentrations and temperatures solutions with spherical micelles form in a first order transition, a transparent, optically isotropic, highly viscous, and elastic cubic phase are observed. The formation of this cubic explained as hard sphere interactions between the aggregates; with farther increasing concentrations transitions to hexagonal and to lamellar phases are observed.
Samples with a smaller hydrophilic EO block, usually forms a hexagonal phases as the first liquid crystalline mesophases. While samples with moderate or high hydrophilic EO block a lamellar phases is found as the first mesophase. These mesophase usually melt at temperature below 100 C.

Micellar solution of tri-block co-polymers have been investigated using many different techniques including: NMR, Static and dynamic light scattering, rheology, fluorescence, using these techniques a lot is known about the properties of the micelles, and as a conclusion measurements obtained by these techniques determine that the core of the micelles consists of PO groups. The core of the micelles is assumed to be free of water, while the EO groups still hydrated.

The solvent quality has a major and controlling factor of block copolymer, while a number for PPO selective solvent have been tested; water is the only selective solvent for PEO block always used. The solvent selectivity of water for PEO and PPO can be altered by a change in temperature or addition of co-solutes such as alcohols or salts any of these factors has great effect on micellar behavior of poloxamer, it is desirable to replace water completely by a non-aqueous polar solvent for some application in which the presence of water is no longer wanted because of many problem such as corrosion possibility.

(Alexandridis, 1998).studied the behaviors of copolymers in non aqueous polar solvent. The considerations have been motivated towards many solvent such as hydrazine, formamide, N-methylformamide, glycerol, propylene glycol, and ethylene glycol. The research indicate the effect of formamide on the tri-block copolymer, since nonionic polymers found to form micelles and even lyotropic liquid crystalline structures in formamide over different conditions more than in water. It was reported that the tri-block copolymer can self assembly in to six different thermodynamically stable microstructure, and at higher co-polymer concentrations a region of hexagonal, bicontinous cubic and lamellar lyotropic liquid crystalline structures are stable. And the stability region of different structures are shifted to higher polymer concentrations and temperature compared to using water as a solvent, suggesting that the effect of effective curvature of the interfaces formed by block co-polymer is higher in formamide than in water.

Alexandridis, et al. 1998 observe The richest structural polymorphism, in equilibrium, in mixtures containing block copolymer, the self assembly of PEO/PPO block copolymer can vary from normal (oil in water) micelles in solution, through all types of normal and reverse (water in oil) lyotropic liquid crystals(normal micellar cubic, normal hexagonal, normal bicontinuous cubic, lamellar, reverse bicontinuous cubic, reverse hexagonal,

reverse micellar cubic), to reverse micelles. The structure in the liquid crystalline phase have been investigated by small angle X-ray scattering (SAXS) and as a conclusion this diverse microstructure of tri-block copolymer is of great importance to numerous practical applications, this ternary system used as a reference in all poloxamer studies relating to phase behaviour

The figure below indicates the ternary phase diagram obtained:

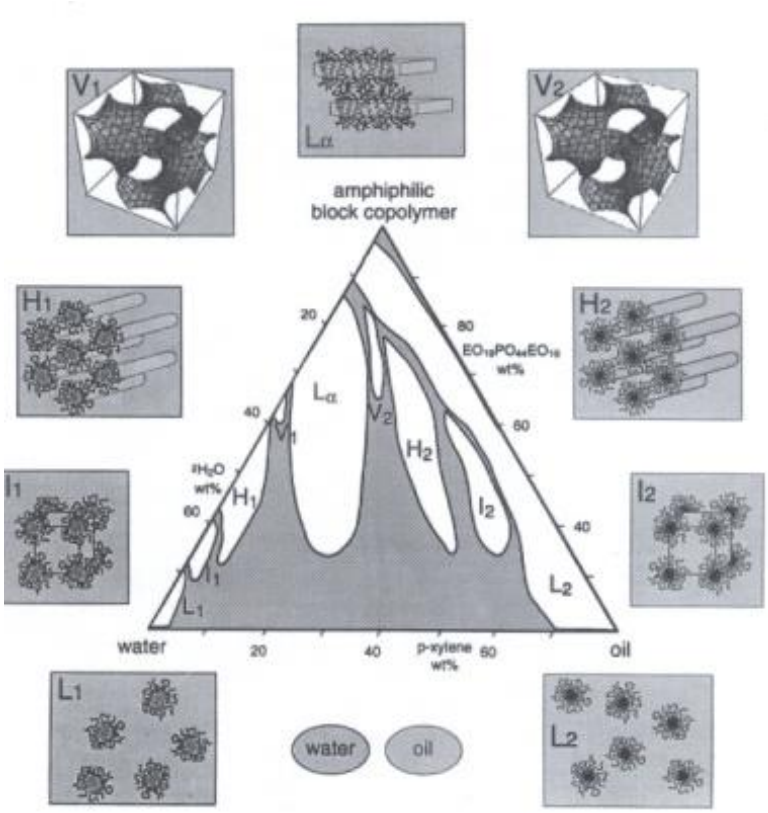

Figure 2.1: A ternary Phase diagram of Tri-block copolymer, Xylene and Water.

Svensson, et al. 1999 explained the result obtained by Alexandridis, et al. 1998 using Lattice mean- field theory model to account for temperature dependent solubility of the blocks consisting the copolymer. The calculation involved free energy determination of the phases obtained in the phase diagram, it was observed that with increasing length of the EO block the reverse phases become unstable; the size increase of the water soluble EO block favors structures with curvature towards polar domain which is normal structure.

Ivanova, et al., presented a useful study of ternary isothermal (25C) systems of pluronic F127 in presence of various co-solvents such as water and polar water miscible solvents like glycerol, propylene glycol and ethanol, and a partially water immiscible solvent like glycerol triacetate and in the presence of surfactants like sodium dodecyl sulphate and cetyl trimethyl ammonium bromide using Small Angle X-ray Scattering (SAXS) addresses the co-solvent/ surfactant effects in the phase behavior of the polymer.

The study indicate that the effects of different co-solvent or surfactant affect the poloxamer phase behavior, it was found that organic solvent, depending on their relative polarity, locate preferably in the PEO rich or the PPO rich domains of the microstructure. Some solvent as ethanol and glycerol triacetate may show amphiphilic behavior and act as co-surfactants and preferably locating at the interface between the PEO rich and the PPO rich domains.

The location of the solvents in the block co-polymer assemblies is established by an analysis of the trends in the structure lattice spacing obtained from SAXS and the interfacial area per block copolymer molecules. In general increasing the solvent polarity will increase the solvent preference to locate in the relatively a polar PPO- rich domains, and hence increases the variety of the microstructure obtained from block copolymer but decreasing their stability range. (Ivanova, et al. 2001).

Frank, et al., studied the properties of mixture of two poloxamer grade of the most popular research focused on the micellization and gellation of two poloxamer F88 and poloxamer P85 in water by use of DSC, SAXS and Rheology. Micellization was mainly characterized by DSC, where as gellation was studied by SAXS, and macroscopic behavior was characterized by Rheology. It appears that micellization was followed by gellation for sufficiently concentrated samples. The results of behavior of Mixture of poloxamer show that the gellation temperature of the poloxamer mixtures was enhanced by more than 10C with respect to gelltaion temperature of the parent poloxamer, so it can be concluded that the mixture composition is an alternative method to modulate the gellation temperature and gel structure, and this behavior seems promising as a model for pharmaceutical application which targeting drug within poloxamer gel.

As the tri-block copolymer show divers polymorphism interesting research published by Kraranikolos, et al. 2008 using stabilized templates of pluronic P105 in the synthesis of optically active semiconductor nanostructures with controlled shape and size. The system exhibit well defined structural order at the nano-scale consisting of spherical, cylindrical or planar microdomains. For the growth of nano-crystals depending in to two steps the first components of the crystals (group II elements) was obtained by introducing the metal by dissolving into the dispersed phase of the templating system and then a hydride gas reacted with pre-composed part of the semiconductor already contained in the block copolymer. These steps lead to nucleate nano-crystal growth inside the block copolymer domains. Coalescence among the domains is slow due to stabilization induced by the long polymeric chains at the interface of each domain and this resulting in long-term stability of the encapsulated nano-crystals. The main critical parameters in nano-materials are particle size, shape, composition and growth environment and all can be controlled by self assembly of tri-block copolymers in selective solvents. Compounds semiconductor quantum dots, nanowires, nanotubes, hollow spheres, and two dimensional nanostructures were grown at room temperature in the self assembled tri-block copolymer.

Karanikolos and Alexandridis reported a techniques for controlled synthesis of ZnSe semiconductor nanostructure by using cubic, hexagonal and lamellar lyotropic liquid crystals as templates, the liquid crystal were formed by self assembly in ternary system consisting of a pluronic P105 amphiphilic block copolymer as the surfactant, heptanes as the non-polar dispersed phase, and formamide as the polar continuous phase.

ZnSe quantum dots and nanowires with diameter smaller than 10 nm were grown incide the spherical, cylindrical and planar nanodomains by dissolving diethylzinc in heptanes domains and reacting the domains with hydrogen selenide gas that brought into contact with liquid crystal in a sealed chamber at room temperature and atmospheric pressure.

Tri-block copolymer are superior candidates component for microemulsion in pharmaceutics application, using microemulsion as a drug delivery system for the purpose of improving the solubility and oral absorption of poorly water soluble drug, has attract much attention in recent years. Microemulsion system has shown to improve absorption of drugs due to small droplet size and promotes intestinal lymphatic transport due to its specific components. Atrovastatin is one of the drug which need to enhance its bioavailability since it is a poor water soluble drug and has a low bioavailability due to rapid metabolism in the gut and liver, since Intestinal permeability is necessary for oral administration so microemulsion as a drug delivery system is promising approach to enhance the solubility and oral absorption of Atorvastatin. A lot work have been done on enhancement of atorvastatin bioavailability.

Surjyanarayan, et al., designed and develop microemulsion drug delivery system of Atorvastatin and investigate it intestinal transport behavior using the single-pass intestinal perfusion method in rat. The permeability behavior of Atorvastatin over three different concentration (10, 20, 40 µg/ ml) was studied in each isolated region of intestine (duodenum, jejunium, ilume and colon) of rat. The human intestinal permeability was predicted using Lawrence compartment absorption model. It was reported that the particle size of microemulsion was 18.3 ± 0.3nm and there was no significant differences between the permeability of the intestinal regions, the estimated human intestinal permeability was relatively higher.

Shun-Ji, J. et al., prepared self-micro emulsifying drug delivery system (SMEDDS) incorporating atorvastatin calcium and evaluate its properties and oral bioavailability. The main droplet size of optimized formulation (20% ethyl oleate, 40% tween 80, 40% Carbitol) was 23.4 ± 1.3 nm. The prepared system appeared to give better dissolution performance studies, compared with raw atorvastain calcium, the release percentage of the prepared system could easily reached more than 95% within 3 minutes which significantly is very high compared to raw atorvastain calcium. The prepared system was relatively stable when stored at 4ºC during 3 months.

Lee, D. et al., prepared self-microemulsifying drug delivery system for oral bioavailability enhancement of Atorvastatin calcium. The release rate of atorvastatin calcium from SMEDDS was investigated in gastric juice at pH 1.2 and intestinal juice at pH 6.8. The concentration of atorvastatin calcium was quantified by high performance liquid

chromatography. The main result f the study were the optimized formulations for in vitro dissolution and bioavailability were capryol 90 40%, cremophor EL 38.7%, and carbitol 19.3 %, the average droplet size distributed was 21.6 nm. The release rate of atorvastatin calcium was higher than conventional marketed tablet.

Pawan, K. et al., formulated a self-emulsifying drug delivery system of Atorvasttain calcium and its characterization including in-vitro and in-vivo studies, The optimize formulation was using Captex 335 as the oil, campul MCM and tween 80 as surfactants mixture, and PEG400 as co-solvent, the best ratio of surfactant was 4:1 wt/wt, the optimize formulation was evaluated for microemulsifying properties and tested for clarity, viscosity, drug content, and in-vitro dissolution and stability study, the formulation significantly reduced serum lipid levels as compared with raw material.

Hai Rong Shen and MingKang Zhong prepared a SMEDDS containing Labrafil as an oil, Cremophor RH40 as surfactant and propylene glycol as co-surfactant, and use it as a formulation to increase the dissolution and permeability of drug by significantly decrease the droplet size to less than 100nm, the prepared SMEDDS might have the potential to improve the oral bioavailability of atorvastatin compared to traditional marketed tablet.

Guohui, et al. recently study the inclusion of Poloxamer 188 as an effective agent in sealing permeabilized cell membranes. In the cases of diseases such as electrical shocks, radiation injuries, and thermal burns which can all lead to a loss of the integrity of the cell membrane which can immediately defect the essential role of the cell membrane as a barrier and affecting its control over the transport of materials into and out of the cell.

Poloxamer 188 was the first in the family of tri-block copolymers to be tested as a candidate for membrane sealant, primarily because it had been widely used in medical, pharmaceutical and cosmetic systems as a solubilizing, wetting and emulsifying agent with low toxicity. To identify the molecular mechanisms of interaction between Poloxamer 188 and damaged membranes, a monolayers phospholipid as a model for the outer leaflet of the membrane naturally found in humans have been treated with Poloxamer 188. Using X-ray and fluorescence microscopy measurements, it was found that Poloxamer 188 changes the phase behavior and morphology of the lipid monolayers. P188 inserts into monolayers phospholipids components and the results indicate that Poloxamer188 insertion is without influencing by head-group electrostatics of phospholipids of the layers. As a result the poloxamer selectively adsorbs into damaged portions of membranes and localizing its effect. This combination of techniques is used for the first time to investigate the lipid/poloxamer interactions as a technique to help the membrane to regain its barrier function so the cell heals and reestablishes its normal lipid packing density.

In another topic of using poloxamer in pharmaceutical application, Hitesh, et al. published that poloxamer 188 has been found to protect against tissue injury in various experimental models by decreases inflammation and tissue damage after experimental brain injury in

rats. A study was made on 6 rats which were treated by poloxamer 188 after experimental injury, the results obtained from the animals assure that Poloxamer 188 has a great effect on preventing inflammation and tissue loss after the injury, these results seems remarkable in using poloxamer as a healing model after medical injury.

New type of study relating about DNA-loaded nanoparticles, done by Csaba, et al., 2006. Its emission is delivering DNA molecule as nanocapsulated forms through cell membrane without any defect or loss using various polymer blends. In vitro cellular uptake plasmid DNA nanoencapsulated in pluronic F68 and other polymer blend particles were studied. In vitro cell culture studies showed that nanoparticles enter the cells and cause transportation of DNA across the membrane. Fluorescent nanoparticles in *in-vivo* studies showed that they are able to overcome nasal mucosal barrier. In the case of immunization studies, DNA-loaded nanoparticles show fast and strong response, more marked than naked plasmid DNA for up to 6 weeks. This shows that these nanoparticles carrier serve to be efficient carriers for delivery of DNA across the nasal mucosal membrane.

With increasing competition and needs for customer compliance, innovations gained a lot of importance in cosmetic delivery systems. Conventional formulations of topical drugs are intended to work on the outer layers of the skin. Typically, such products release their active ingredients upon application, producing a highly concentrated layer of active ingredient that is rapidly absorbed, providing a short duration of action but more serious side effects can occur when active ingredients penetrate the skin as irritation and allergic reactions and burning in significant users.

The application of topical drugs has many problems like greasiness, stickiness associated with the ointments that often result in lack of patient compliance. All of these complications could be overcome by using a unique, versatile and novel approach microemulsion in drug delivery system, especially when dealing with sensitive active material as Hydroquinone.

A lot of research focused on using micosponge approach for delivering Hydroquinone for topical use. Microsponge technology allows an even and sustained rate of release, reducing irritation while maintaining efficacy. (Saraf, et al, 2012).

SkinMedica Incorporation manufacture and market a EpiQuin Micro, which is a product based on Microsponge system uses microscopic reservoirs that entrap hydroquinone and retinol. The Microsponge delivery system releases these ingredients into the skin gradually throughout the day. This provides the skin with continuous exposure to hydroquinone and retinol over time. (Grimes, et al, 2004).

Meraz prepared a formulation of HQ 4% with retinol 0.15% entrapped in micro-sponge reservoirs by two steps polymerization process in which Eudragit RS 100 was dissolved in ethyl alcohol. Then, drug added to solution and dissolved under ultrasonication at 35 C. The inner phase was poured into the PVA solution in water (outer phase). Following 60 min of stirring, the mixture is then filtered to separate the microsponges. The system was developed to release Hydroquinone gradually to prolong exposure to treatment and to minimize skin irritation. The safety and efficacy of this product were evaluated in a 12-week open-label study. A total of 25 patients completed the study. Study end points included disease severity, pigmentation intensity, lesion area, and colorimetry assessments.

Adverse events also were recorded. Patients applied the microentrapped Hydroquinone 4% formulation to the full face twice daily (morning and evening), Patients were evaluated at baseline and at 4, 8, and 12 weeks. The microentrapped Hydroquinone 4%/retinol 0.15% formulation produced improvement at all study end points, the preparation was safe and effective.

Lin, et al, formulated an anhydrous emulsion system, without the aqueous phase, using deoxyArbutin, decomposes to hydroquinone in aqueous solutions.
An anhydrous emulsion system could offer a stable vehicle for compounds that are sensitive to hydrolysis or oxidation. Therefore, to enhance the stability of deoxyArbutin in formulations, polyol-in-silicone as the basic formulation for investigation. The quantity of deoxyArbutin and the accumulation of hydroquinone at various temperatures analyzed through an established high performance liquid chromatographic (HPLC) method.
The results indicated that water increased the decomposition of deoxyArbutin in the formulations and that the polyol-in-silicone, oil-based, anhydrous emulsion system provided a relatively stable surrounding for the deoxyArbutin that delayed its degradation at 25 °C and 45 °C. Moreover, the composition of the inner hydrophilic phase, containing different amounts of glycerin and propylene glycol, affected the stability of deoxyArbutin. the percentage of hydroquinone was calculated by the molar ratio of deoxyArbutin to Hydroquinone as 3 g of deoxyArbutin can completely decompose to 1.701 g of hydroquinone.

Hans-Jorgebnu and Eckhard prepared Hydroquinone aqueous base using α, ß-cyclodextrin complexes, due to the ability of cyclodextrin complexes of prevention the oxidation of hydroquinone and offer greater stability. The results confirm that Hydroquinone cyclodextrin complexes offer a greater depigmentaion more than hydroquinone itself enhances skin- whitening effect. cyclodextrins derivatives are not a nutrient medium for microorganisms, As a result, the use of preservatives in formulations can be reduced, This is a further advantage of preparations.

Chapter Three
Aims of the Study

Chapter three
Aims of the study

1. Development and characterization an alcohol-free microemulsion using promising tri-block co-polymer Poloxamer 188: polypropylene oxide polyethylene oxide polypropylene oxide ($HO(C_2H_4O)_{80}(C_3H_6O)_{27}(C_2H_4O)_{80}H$) as a main surface active agent and other safe component.

2. Developing and studying the phase behavior of tri-block co-polymer as main surfactant and in combination of co-surfactants/ co-solvent, with water.

3. Develop the main phase behavior diagrams of tri-block co-polymer and with combination of co-surfactants/ co-solvent.

4. Investigate the effect of addition of co-surfactants/ co-solvent on phase behavior of system containing tri-block co-polymer.

5. Investigate the effect of temperature of the obtained phase behavior of tri-block co-polymer.

6. Use the prepared microemulsion in pharmaceutical application system to enhance the bioavailability of Atorvastatin calcium a poorly water soluble drug.

7. Use the prepared microemulsion in cosmetics industry as suitable system to prepare Hydroquinone gel.

8. Obtain the optimum microemulsion formulation to be further investigated on stability program.

9. Investigate the optimum microemulsion formulation at accelerated stability conditions.

10. Evaluate the stability results of the obtained system.

Chapter Four
Materials and Method

Chapter four
Materials and method

4.1 Materials

Chemicals components used in this research for microemulsion preparation selected based on many properties as safety, compatibility, availability, ease of preparation and stability.

Poloxamer 188 used as tri-block co-polymer surfactants for this research, the sample was donated by BASF Corp. (Israel) and was used as received from the manufacturer without any further Investigations.

Lime oil samples were purchased from Frutarum Company (Haifa, Israel), the received samples analyzed for physical properties and results conforms to the manufacturer specification.

Cremophore RH 40, polyoxyl 40 hydrogenated castor oil.
The samples of Cremophor RH 40 purchased from BASF Corp. (Israel), the samples used as received without further investigation.

Tween 80, Polyoxyethylene 20 sorbitan monooleate, Polyoxyethylene sorbitan fatty acid esters. The samples of Tween 80 purchased from Sapo Company (Israel) and used as received without further investigation.

Propylene Glycol: the samples purchased from Gadot Company (Israel) and used as received without further investigation.

Hydroquinone: samples of active material purchased from Parchem Company (USA), the content of the drug was 100.0 %.

Atorvastatin Calcium: samples of active material purchased from Jiangsu Haohan Chemical Co.,Ltd (China), the content of the drug was 100.5 % (Relative to standard).

Butylated hydroxylToluene (BHT); samples purchased from Nova International Company (India), and used as received without farther investigation.

Sodium Metbisulfite: Samples purchased from Sigma-Aldrich (Isreal) and used as received without farther investigation.

Distilled water obtained from Quality Control lab of Jerusalem Pharmaceuticals.

4.2 Instruments and equipments

Analytical balance (Acculab Vicon Vic-303).

HPLC; Merck Hitachi Lachrom Elite HPLC System.

Column: Octyl silane chemically bonded to totally porous silica, 25 cm length and internal diameter of 4.6 mm.

Vortex mixer (mrc Laboratory Equipment VM-1000).

Brookfeild Visvometer (Model A serial number 8534655, Massachusetts, 02346 USA).

Polarizer microscope (Olympus).

conductivity meter LF 538 (WTW).

Refractometer mrc K7135.

Incubator Feirrlaboo FI 177.

Refrigerator RF 675.

pH meter: Hanna Instrument HI 1771.

Cross polarizer, Shaker, Water bath, 10 mm glass test tubes with screw caps, Sonicator, Glass Beaker, Volumetric Flasks, Volumetric and graduated Pipettes.

4.3 Procedure

4.3.1. Constructing ternary phase diagram

Sample of Tri-block copolymer surfactants

Samples were prepared by weighing appropriate amounts of each component into 10 mm glass test tubes with screw caps at ambient condition. The volume of water was added drop wise by titration then the samples were mixed by vortex then stand until equilibrate. Physical properties as clarity, color and number of phases obtained were detected organoleptically. Polarized microscope was used to detect anisotropy. Finally draw the phase diagram using OriginPro 7.5 program develop by OriginLab Corporation. Each set of samples for each phase diagram system prepared with same procedure then the work was done with the same procedure and at 37°C.

4.3.2. Application in Pharmaceutical Formulation:

The constructed phase diagram of the studied system used to prepare formulation to enhance oral bioavalabilty of Atrovastatin poorly water soluble drug. Atorvastatin introduced to combinations of defined one phase region obtained in the constructed phase diagram for different systems.

4.3.3. Application in Cosmetics Formulation:

The constructed phase diagram of the studied system used to prepare formulation to enhance solubility and stability of Hydroquinone.
Hydroquinone active material introduced to a combination of defined one phase region obtained in the constructed phase diagram.

4.4 Stability Study:

4.4.1. Accelerated stability study;

The prepared samples studied at accelerated stability program the samples have been studied under stressed condition of temperature and Relative Humidity.
The most common main stress conditions for stability indicating technique: are 40 ± 2ºC, 75± 5% RH for all dosage forms and 5 ±3 ºC for gel and solutions dosage forms.

For pharmaceutical preparations the samples studied at 40 ± 2ºC, 75 RH for 3 months. The samples analyzed at time of preparation as zero time and at the end of the period.

The Cosmetics preparations the samples studied at 40 ± 2ºC, 75 RH relative humidity for 14 days. The samples analyzed at time of preparation as zero time and at the end of the period.

4.4.2. Ambient stability study;

Another set of prepared samples from cosmetics and pharmaceutical preparations studied at ambient conditions at room temperature for defined period of time exceeding 1 month.

4.4.3 Determination of Drug Content:

The content of atorvastatin determined by using HPLC chromatography within the following conditions:

The chemical reagent used is: Acetonitrile, methanol, glacial acetic acid and water.

Mobile Phase:

The mobile phase prepared using a mixture of methanol, 1% (v/v) glacial acetic acid and acetonitrile (60:30:10).

The Standard Preparation:

Prepared by weighing a quantity of Atorvastatin calcium working standard equivalent to about 100 mg of Atorvastatin. Transfer to a 100-ml volumetric flask, complete to volume with methanol and mix. dilute 5 ml of this solution to 100 ml with water and mix.

The sample Preparation:

The test samples prepared by transferring a quantity of preparation equivalent to about 100 mg of Atorvastatin to a 100-ml volumetric flask, complete the volume with methanol. Then 5 ml of this solution diluted to 100 ml with water.

The proper chromatographic conditions:

Detection Wavelength: 254 nm.

Column: Octyl silane chemically bonded to totally porous silica, 25 cm length and internal diameter of 4.6 mm.

Flow Rate: 1.5 ml/min.

Injection volume: 20 µL

Testing Procedure:

Both the Standard Preparation and the Assay Preparation were separately injected into the Liquid Chromatograph. The chromatograms were record and the responses for the major peaks were measured.

Finally the percentage of Atorvastatin calculated using the following formula:

$$\% \text{ Atorvastatin} = \frac{\text{Area Under Peak of Assay Prep.}}{\text{Area Under Peak of Standard Prep.}} \times 100\%$$

4.5. Methodology:

To clarify and understand the behavior of Poloxamer 188 with lime oil, a phase diagram was prepared. Samples of poloxamer 188 and lime oil covering all the wanted area of phase diagram were weighted and drop wise water titration method used to reach the final volume. After each addition the samples were left for equilibrium time. Then the samples were carefully optically investigated for physical changes during the study period. Each sample was checked for phase separation, the phase diagram prepared depending on the data obtained after the end of study period. Just the one phase areas were plotted on the phase diagram and constructed for farther investigation. Areas with multi phases were not included in this study. Each system took 3-4 weeks to be finished.

To study the phase behavior of the tri-block copolymer with co-solvent, another set of samples after introducing Propylene glycol were prepared and the same procedure were followed.

A process have been taken in order to clarify and characterize the behavior of the tri-block copolymer in presence of another surfactant, a third set of samples after introducing tween 80 an a co-surfactant were prepared and the results were obtained by the same procedure.

A Fourth phase diagram system was prepared using another nonionic surfactant as co-surfactant, a fourth set of samples after introducing cremophore RH40 were prepared and obtaining the phase diagram including clarify regions by the same procedure. As an effective application this phase diagram used to prepare a miceroemulsion system to enhance the solubility of atorvastatin calcium, and the prepared samples were studied under stability study.

And finally for pharmaceutical application the effect of temperature on the phase behavior of the tri-block copolymer surfactant was investigated. The system of fourth phase diagram obtained after introducing Cremophore RH40 was investigated on 37ºC, the results were obtained and a final phase diagram was plotted.

The studied system used to prepared gel and solution of Hydroquinone aqueous base for topical use, the optimized formulations were determined and further examination were constructed.

Chapter Five
Results and Discussion

Chapter five
Results and discussion

The component of each system have been chosen depending on many factors . oil was chosen as a best solvent for Active ingredients. Propylene glycol was chosen for its better solubility enhancement for Atorvastatin calcium more than polyethylene glycol 400 or glycerin. And as known for propylene glycol, it has a conditioning effect on skin and work as penetration enhancer. The other non ionic surfactants as Tween 80 and Cremophore RH 40 known for their safety, compatibility, stability and availability.

5.1 Constructing the Phase diagram

The phase diagram of ternary component obtained for each system as the following:
The ternary phase diagram of poloxamer 188, water and lime oil at room temperature presented in figure (5.1).

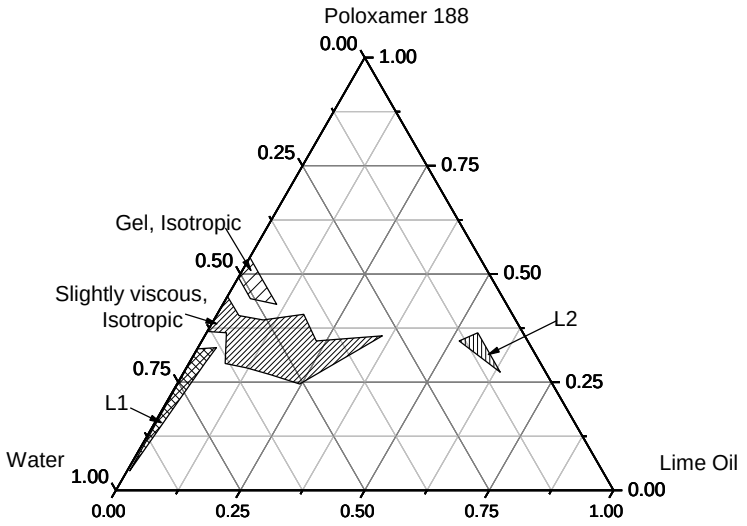

Figure 5.1: Ternary Phase diagram of three main components: Poloxamer 188, Lime oil and water, the study obtained at room temperuture.

As appears on Figure (5.1) the phase diagram of four one phase regoins were obtained, these regoins were clear homogeneous and isotropic under polarized light. The main differece betweeen them were in physical properties especialy the viscosity. It was clearly varied from one reagoin to another.

The L1 regoin appears almost on the binary line between poloxamer and water mostly to the water rich reagoin, the phase is isotropic under polarized light.
The L2 regoin appears on the oil rich phase it was isotropic under polarized light its conductivity value was 9.6 µS.

The slightly viscous regoin appears in the middle of the phase diagram was clear isotropic under polarized light its conductivity value was 37.5 µS, as the boundries of this regoin appears on the binary line for poloxamer and water at below 50 % poloxamer content, and

depending on the isotropic properites, viscousity, conductivity and the position in phase diagram it is suggested to be normal Cubic Micellar [I_1] regoin.(Alexandridis, et.al, 1998).

The gel phase appears at aproximitly 50% of polymer concentration, it was isotropic under polarized light clear stif gel phase, according to its position and depending on the behaviuor of poloxamer and water on the binary line between water and poloxamer it is suggested to be normal Hexagonal phase, as it is known the hexagonal phase is shiny and anisotropic under polarized light, but these properties do not obtained within the investigated gel sample, it can be justificated as the poloxamer is a weak nonionic surfactant and consist of blocks building units it is hard to show anisotropic properties especially when intorducing the oil, this led to loss the structure of Hexagonal make it more difficult to be identified by simple polarized light.

The phase diagram of pluronic F127 with xylene constructed by Alexandridis, et.al. 1998 indicated that the H_1 phase apears after micellar cubic regoin as the poloxamer concentration increases,this note can help in clarifing the gel regoin. Also the gel phase appears at high poloxamer concentration (more than 50 %) and that agree with Wanaka, et al. 1999 results which indicated that at higher concentrations solutions with spherical micelles form in a first order transition, a transparent, optically isotropic, highly viscous, and elastic cubic phases are observed. The formation of this cubic explained as hard sphere interactions between the aggregates; with farther increasing concentrations transition to hexagonal and to lamellar phases occurred.

As an effective step to enlarge the one phase regoins obtained in the prevois phase diagram appeared in Figure (5.1) propylene glycol added to lime oil with 50% in concentration, the effect of propylene glycol on the behavoir of the poloxamer 188 seems in the figure (5.2).

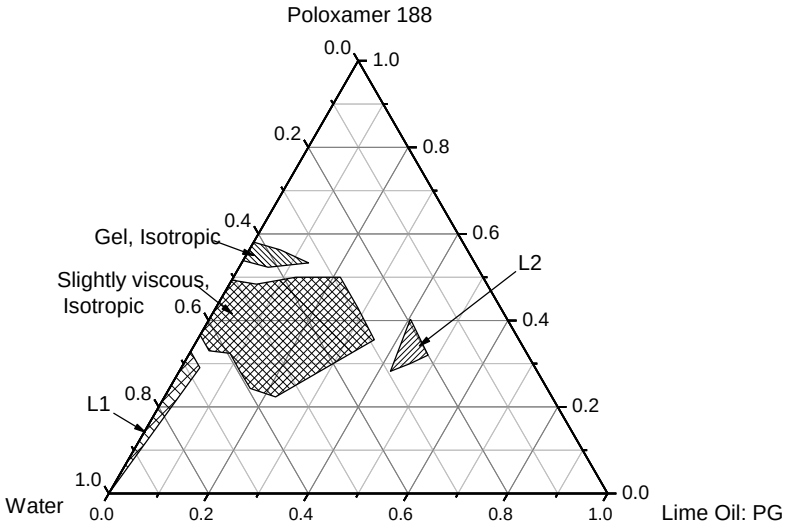

Figure 5. 2: Ternary Phase diagram of three main components: Poloxamer 188,Oil Mixture (Lime oil: Propylene glycol 50:50) and water, the study obtained at room temperuture.

A four one phase homogenous clear and isotropic regions obtained in Figure (5.2). The effect of propylene glycol appears in the phase diagram by spreading the slightly viscous phase to be the largest region and larger than the phase obtained in the previous system obtained in figure (5.1).

The liquid phase L_1, the gel phase, the slightly viscous phase and L_2 phase all these phases still obtained in the phase diagram appears in figure (5.2). As comparing the conductivity values of the obtained phases; the L_2 conductivity value at room temperature was 11.6 µS and the slightly viscous region was about 50.5 µS, this shift in the conductivity values indicating the excess presence of water as transferring from L_2 in oil rich corner to water rich corner and that confirm the effect of propylene glycol on the behavior of poloxamer.

Depending again on the phase diagram of Pluronic F127 done by Ivanova. et. al. 2006 with propylene glycol, the phase diagram of Pluronic F127 and propylene glycol indicate the presence of three main phases the largest phase was I_1 which exist between 18 to 65 % approximately of Pluronic F127 and H_1 region between 68 – 78% of Pluronic F127. These results may help in identifying the gel phase and slightly viscous region as pluronic F127 has 70% ethylene oxide and Poloxamer 188 has 80% of ethylene oxide, these notes may conclude to support the suggestions of these unknowns regions.

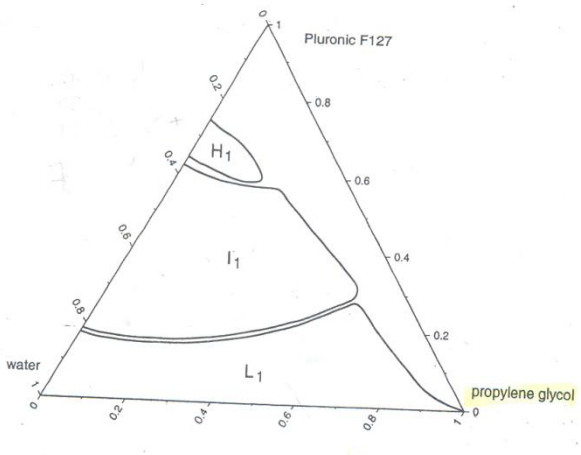

Figure5.3: Ternary Phase Diagram of Pluronic 127, Propylene glycol and water

When this was system used to solubilize atorvastatin calcium, the results were not so good The poloxamer alone could not give stable formulations. It seems helpful to add another surfactant to poloxamer 188, to enhance the preparation of stable and useful formula to be used as superior formulation to enhance the stability, solubility and bioavailability of poorly soluble drugs. Tween 80 is a non ionic surfactant and pharmaceutically compatible component to be used in pharmaceuticals, cosmetics and in food products. So Tween 80 was introduced to the system and the phase behavior of the components studied , the results of the system appears in the figure (5.4).

Ternary phase diagram of mixture of surfactant contains 75% of Poloxamer 188, 25% of Tween 80, oil mixture contains 50% lime oil and 50% propylene glycol obtained at room temperature a three clear one phase regions obtained as illustrated in figure (5.4).

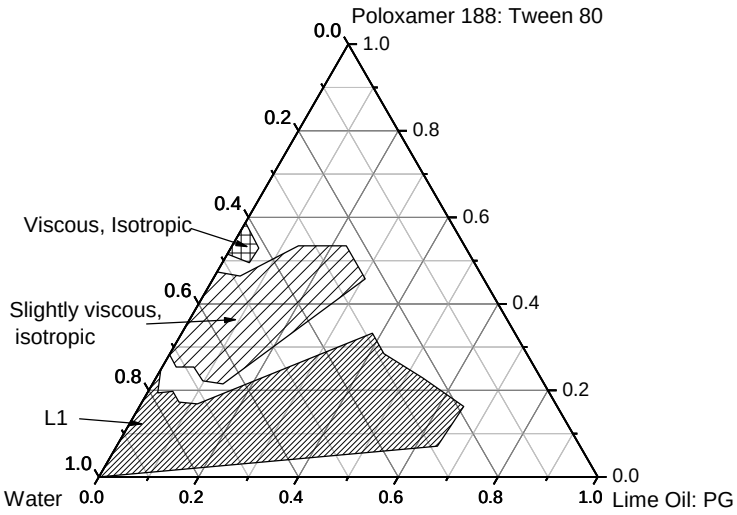

Figure 5. 4: Ternary Phase diagram of three main components: Surfactant Mixture (Poloxamer 188 : Twen 80,75: 25), Oil mixture (Lime oil: propylene glycol 50:50) and water, the study obtained at room temperuture.

The main results were three homogenous clear and isotropic under polarized light one phase regions the narrow viscous phase, the intermediate slightly viscous phase and the wide L_1 liquid phase, the liquid phase on water rich corner is Micelle phase L_1 its conductivity value was 21.3 µs it was not viscous and isotropic.

Tween 80 work as co-surfactant with percentage of 25%, the percentage of Poloxamer 188 was 75% from the total amount of surfactant; it helps in increasing the solubilization capacity of the system, as appears in figure (5.4) much oil solubilized in the formulation more than in using poloxamer 188 alone.

When using the system for preparation of Atorvastatin microemulsion, the obtained samples were not stable. Tween 80 forms cores emulsions which affect the shelf life and finally separation occurs. The gel phase does not appear in the figure that because the study includes the clear one phase regions only and exclude the multi phases regions.

Another surfactant was used instead of Tween 80, a system using Cremophore RH40 co-surfactant with the same ratio as in case of Tween 80, the phase diagram appears in figure (5.5) illustrate the result.

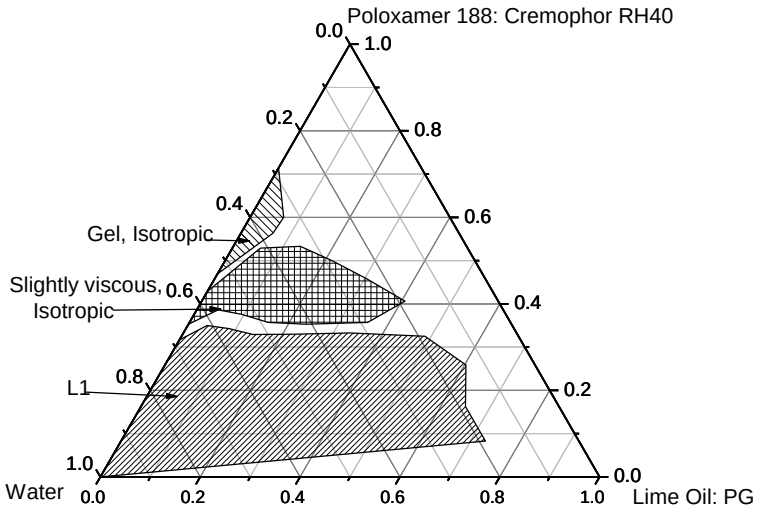

Figure 5.5: Ternary Phase diagram of three main components: Surfactant Mixture (Poloxamer 188: Cremophore RH40 75:25), Oil Mixture (Lime oil: propylen glycol 50:50) and water, the study obtained at room temperuture.

Figure (5.5) shows a three main clear homogenous isotropic one phase regoins, using Cremophore RH40 increasing the solubilzation of oil more than in the case of Tween 80, escpically in L_1 phase the water rich regoin. The conductivity values were 25.4 µs and 16.4µs for L_1 and the slightly viscous regoins, respictivly, these results indicating an excess in the presence of water as transfereing from water lean to water rich regoins in the phase diagram.

Here the gel phase appearse again in the phase diagram,and again the slightly vicous regoin is suggested to be normal Cubic (I_1) phase and the gel is suggested to be normal Hexagonal (H_1) phase depending on the referece phase diagram of Pluronic F127 with xylene, and the peroperties of these regoins.

In order to identify and characterize the effect of temperature on the phase behavior of the prepared mixture of surfactant and co-surfactant, Oil mixture in the last composition of figure (5.5), the system studied at 37 ºC and the phase diagram obtained illustrated in figure (5.6):

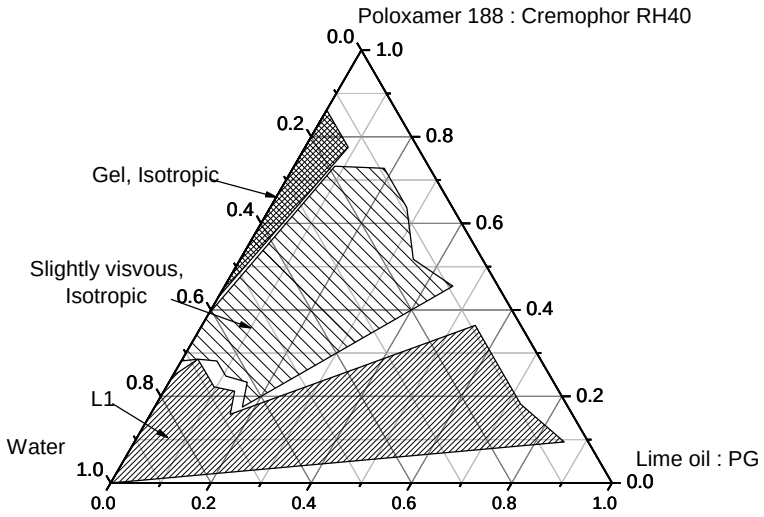

Figure 5.6: Ternary Phase diagram of three main components: Surfactant Mixture (Poloxamer 188: Cremophore RH40 75:25), Oil Mixture (Lime oil: propylen glycol 50:50) and water, the study obtained at 37C.

The phase diagram in figure (5.6) appears three isotropic one phase regions as in figure (5.5) It is clear that the main difference in the two diagrams is the spreading of one phase regions to oil rich regions and that confirmed by the effect of temperature on non ionic surfactant.

Figure (5.6) phase diagram indicates the behavior of poloxamer 188 and the effect of addition of co-solvent and co-surfactant to its behavior, and the effect of temperature was clearly obtained, increasing the temperature the solubility of surfactant in oil increases this results clarified by a large spreading of L_1 phase isotropic region comparing with the L_1 phase in figure (5.5). All the one phase regions were isotropic under polarized light, and vary in viscosity.

As comparing the results with the binary phase diagram of Poloxamer 188 appears in a published work for Wanaka, et al. 1994, there is a kind of compatibility of the results.

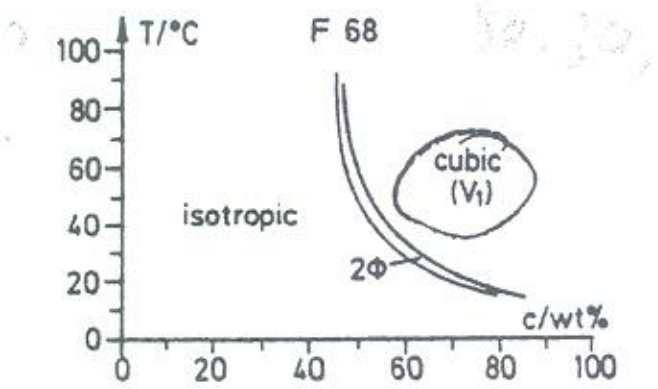

Figure 5.7: phase diagram of the system Pluronic F68 [adapted from Wanaka, et al.1994]

The system shown above in figure (5.7) indicates the relation between increasing concentration of tri-block copolymer and temperature. The diagram indicates tow main regions the isotropic regions cover the largest area of the curve and the cubic V1 region appears on high polymer concentration at low temperature range.

In a comparison with the results obtained by this research and the binary phase diagram appeared in figure (5.7) , there is a kind of compatibility between the results in obtaining the isotropic region of polymer at concentration up to 75% and at about 40 C , in which for this research the room temperature nor the stability study would not exceed this temperature, all the system studied in this research the isotropic regions obtained, and when introducing the co-solvent or co-surfactant to the system it changes the preference of poloxamer especially in the boundaries of the obtained region but the main isotropic region obtained in all of them with differing in physical properties specially the viscosity. Of course the system studied in this research differ on its component from figure (5.7) phase diagram of binary system of Pluronic F68 but it is seems to be good to compare the results with known behavior of the same tri block co-polymer.

The Cubic V1 region obtained in the system Pluronic F68 obtained at polymer concentration start at about 50% on temperature exceeding 90C is a remarkable result its main properties is anisotropic under polarized light and has a high viscosity, this region is not obtained on the studied systems in this research.

The main regions obtained from the systems studied in this research a clear L_1, L_2, normal cubic I_1, and normal Hexagonal H_1. The identification of hexagonal was slightly complicated, as introducing many components on the system with poloxamer the structure of hexagonal was loss, a kind of melting of the stiff hexagonal structure occurred which make it difficult to identify the anisotropic properties under simple polarized light and more developed techniques have to be used as Small Angle X-ray Scattering (SAXS), NMR studies which are not available at the Lab where this work have been constructed.

5.2. Application in Pharmaceutical Formulation:

Enhancement bioavailability of Atorvastatin:

For Pharmaceuticals applications the prepared system used to enhance the solubility and bioavailability of poorly water soluble and low oral bioavailability of Atorvastatin. Atorvastatin calcium used as salt form of active ingredient as it's the known form of active material in oral tablet.

5.2.1. Pre-formulation:

A series of formulation were prepared using lime oil as solubilizer, Poloxamer 188 as a main surfactant and propylene glycol as co-solvent and Tween 80 and Cremohpor RH40 as co-surfactants. The prepared formulation listed in table (5.1):

Table (5.1-A): Composition for various formulations containing Atorvatstain.

Component (w/w %)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Atorvastatin Calcium	0.49	0.63	0.59	0.66	1.54	1.71
Lime Oil	9.3	9.32	23.53	16.56	30.77	34.19
Propylene glycol	9.3	9.32	23.53	16.56	30.77	34.19
Poloxamer 188	31.1	30.43	8.24	23.18	10.77	11.97
Tween 80	0	0	0	0	0	0
Cremophor RH40	12.4	13.03	35.29	9.93	4.62	5.13
Water	37.4	37.27	8.82	33.11	21.53	12.81

Table (5.1-B): Composition for various formulations containing Atorvatstain.

Component	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**
Atorvastatin Calcium	1.4	1.2	0.80	1.6	4.8	1.4
Lime Oil	7.0	14.7	31.7	31.5	11.9	16.4
Propylene glycol	7.0	14.7	31.7	31.5	11.9	16.4
Poloxamer 188	42.3	20.6	11.9	11.8	17.9	32.9
Tween 80	0	0	4.0	3.9	6.0	0
Cremophor RH40	14.1	8.8	0	0	0	0
Water	28.2	40	19.8	19.7	47.6	32.9

Table (5.1) describe 12 formulation prepared as a main formulation for Atorvastatin, all were prepared simultaneously and at same conditions of temperature and humidity.

5.2.2. The Specification of the Finished Product:

The prepared sample ranging from F1 to F12 prepared at room temperature the specification and results illustrated in table (5.2):

Table (5.2-A): Results at Time of Preparation (Zero Time).

Test	**Limit**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Color	Colorless - yellowish	Conform	Conform	Conform	Conform	Conform	Conform
Odor	Lime	Lime	Lime	Lime	Lime	Lime	Lime
Clarity	Clear	Clear	Clear	Clear	Turbid	Turbid	Turbid
Appearance	Solution	Solution	Solution	Solution	Solution	Solution	Solution
pH	6.0 – 8.0	6.8	6.7	6.8	6.8	6.8	6.8
Viscosity	NMT 10000 cps	1700	1500	2000	2000	2500	1200
Date		8\12	8\12	7\12	7/12	5\12	7\12

Table (5.2.B): Results at Time of Preparation (Zero Time).

Test	**Limit**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**
Color	Colorless - yellowish	Conform	Conform	bluish	bluish	bluish	white
Odor	Lime	Lime	Lime	Lime	Lime	Lime	Lime
Clarity	Clear	clear	Turbid	clear	clear	Turbid	Turbid
Appearance	Solution	Solution	Solution	Solution	Solution	Solution	Suspension
pH	6.0 – 8.0	6.8	6.8	6.8	6.8	6.8	6.8
Viscosity	NMT 10000 cps	25000	2000	500	500	1200	500
Date		8\12	8\12	7\12	7/12	7\12	7\12

As Appeared in table (5.2) some of the prepared formulation fail to obtain the main specification of the finished product, specially the physical appearance, F4, F5, F6, F8, F11 and F12 for turbid solution which will lead to precipitation after a period of time so these formulation were excluded.

5.2.3. **Stability Result at Ambient Condition:**

The prepared formulation stored at room temperature and the result illustrated in the tables below:

Table (5.3-A): Stability Results at Ambient Conditions (Room Temperature).

Test	Limit	F1	F2	F3	F4	F5	F6
Color	Colorless - Yellowish	Conform	Conform	Conform	Conform	Conform	Conform
Odor	Lime	Lime	Lime	Lime	Lime	Lime	Lime
Clarity	Clear	Clear	Clear	Turbid	Turbid	Turbid	Turbid
Appearance	Solution	Solution	Solution	Solution	Suspension	Suspension	Suspension
pH	6.0 -8.0	6.8	6.7	6.8	6.8	6.8	6.8
Viscosity	NMT 3000 cps	1500	1300	1800	1700	3000	1000
Date		11\12	10\12	10\12	9\12	9\12	10\12

NMT: Not More Than.

Table (5.3-B): Stability Results at Ambient Conditions (Room Temperature).

Test	Limit	F7	F8	F9	F10	F11	F12
Color	Colorless - Yellowish	Conform	Conform	Conform	Conform	Conform	Conform
Odor	Lime	Lime	Lime	Lime	Lime	Lime	Lime
Clarity	Clear	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
Appearance	Solution	Solution	Suspension	Solution	Solution	Suspension	Suspension
pH	6.0 -8.0	6.8	6.8	6.8	6.8	6.8	6.8
Viscosity	NMT 3000 cps	27000	1700	700	600	1300	300
Date		11\12	11\12	9\12	9\12	11\12	11\12

Table (5.3) describe the results of formulations stored at room temperature after a period of time ranging from 2 -3 months, the formulations numbered as F3, F4, F5, F6, F7, F8, F9, F10, F11 and F12 show significant change in the physical properties of the finished product, so these set of formulation could not be constructed to accelerated stability study and have to be excluded.

5.2.4. Accelerated Stability Study:

Samples from F1 and F2 studied at 40 ± 2C; RH 75% for 3 months, there was no significant change during the mentioned study period.

Table (5.4): Stability Results at Accelerated Conditions.

Test	Limit	0 Time		3 months	
		F1	F2	F1	F2
Color	Colorless - Yellowish	Con.	Con.	Con.	Con.
Odor	Lime fragrance	Lime	Lime	Lime	Lime
Clarity	Clear	Clear	Clear	Clear	Clear
Appearance	Solution	Sol.	Sol.	Sol.	Sol.
pH	6.0 - 8.0	6.8	6.7	6.8	6.8
Viscosity	NMT 10000 cps	1500	1300	1400	1200
Assay: Atorvastatin calcium	90.0 – 110.0 %	105.0 %	97.0 %	103.0 %	98.0 %
Degradation	NMT 2.0 %	UN	UN	UN	UN

NMT: Not More Than. UN: Undetected: below detection limit.

Table (5.4) describe the stability results of Formulation numbered as F1, F2 studied at accelerated stability of temperature and relative humidity for 3 months, the result indicate there is no significant change in the physical and chemical properties within the mentioned study period.

5.2.5. Stability at Refrigerator 5 ± 3ºC:

The prepared samples stored at temperature range of 2-8C and the results illustrated in the table below:

Table (5.5): Stability Results at Refrigerator.

Test	Limit	0 Time		3 months	
		F1	F2	F1	F2
Color	Colorless - Yellowish	Conform	Conform	Conform	Conform
Odor	Lime fragrance	Lime	Lime	Lime	Lime
Clarity	Clear	Clear	Clear	Clear	Clear
Appearance	Solution	Solution	Solution	Solution	Solution
pH	6.0 - 8.0	6.8	6.8	6.8	6.8
Viscosity	NMT 10000 cps	1500	1300	1100	1000
Assay: Atorvastatin calcium	90.0 – 110.0 %	105.0 %	97.0 %	103.0 %	98.0 %
Degradation	NMT 2.0 %	UN	UN	UN	UN

NMT: Not More Than. UN: Undetected: below detection Limit.

5.2.6. Content of drug:

As atorvastatin microemulsion dosage forms not established by any pharmaceutical reference the content limits were in housed designed to be 90.0 to 110.0 % of the labeled content, all the results were within the designed range.

In order to examine the effectiveness of test method a blank sample of the formulation were prepared without the active pharmaceutical ingredient and analyzed within the same procedure the results were complies with what expected, the peak of atorvastatin was not obtained on the chromatogram for blank samples.

5.2.7. Identification Test:

For identification and validation purposes the chromatograms obtained from prepared samples were compared, there were compliance between the samples of standard preparation and the test preparation. The following figures identify the obtaining chromatograms:

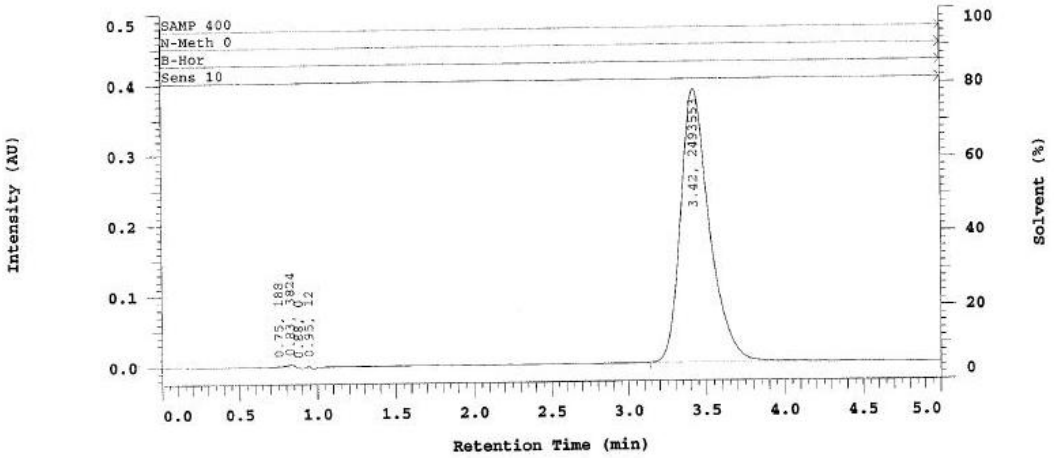

Figure (5.8): the chromatogram of standard preparation.

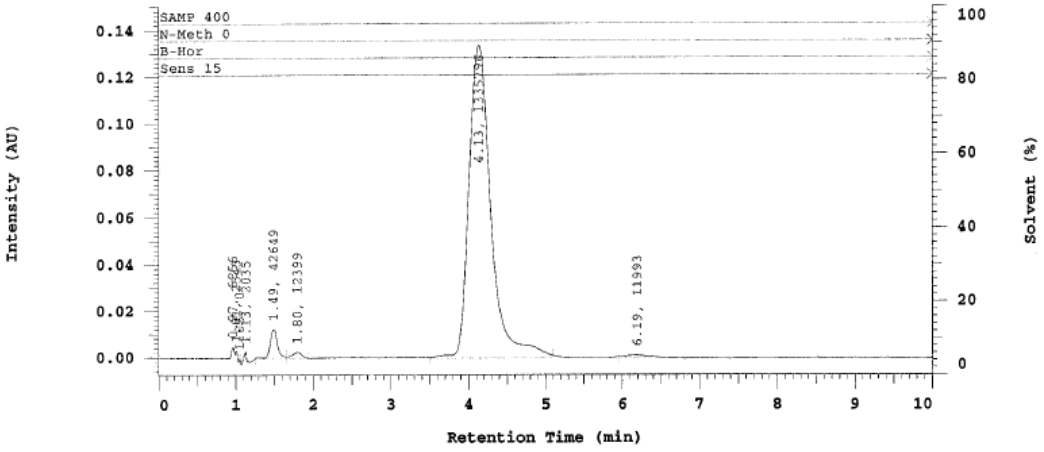

Figure (5. 9) : the chromatogram of Sample preparation.

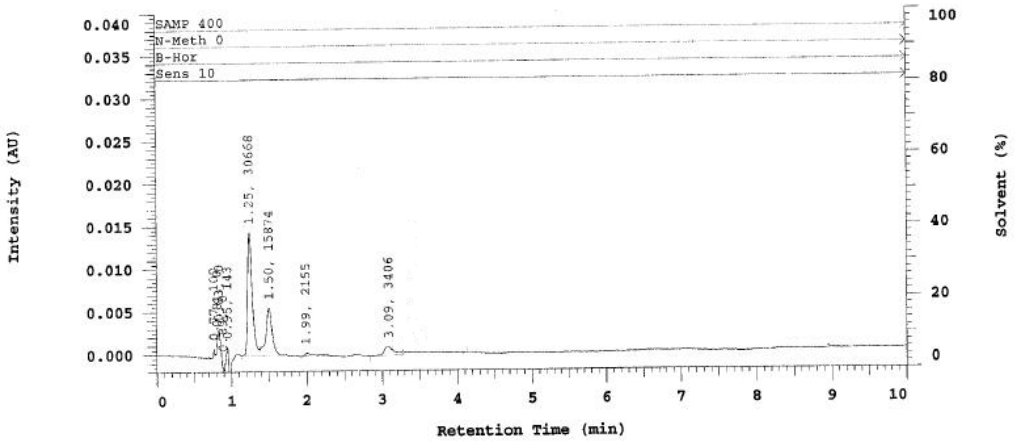

Figure (5.10) : the chromatogram of blank preparation.

As appear in the obtained chromatograms the retention time of Atorvastatin peak in the chromatogram of the sample Preparation corresponds to that of the Standard Preparation and there are no peaks on the same retention time on the blank preparation.

The noise peak appears in the beginning of the chromatogram treated as unreturned and have no effect on the values of area under the main peaks of the active ingredient and this result is very important for validation studies which means that the supplied test method in selective for the active ingredient.

5.2.8. pH Test:

The limit of pH has designed in house to be (6.0 – 8.0)

The pH = 6.8 on zero time and at the end of the mentioned study period there was no significant change on the pH value of Atorvastatin microemulsion.

As mentioned ealier the formulation have to have a pH value above 4.5 at least 5.5 which is higher than pKa of Atorvastatin. The preparation formulations has pH value 6.8 and maintain within the same range, it is a great benefit and seems promising in bioavailability enhancement.

5.2.9. Dilution study:

The prepared samples tested for dilution using different solvent: water, 0.1 N HCl, 6.8N Phosphate buffer, the results illustrated in table below:

Table (5.6): Stability Results for dilution study at Room Temperature.

Test:	Limit	F1	F2
Dilution with H_2O (1ml to 100ml)			
Color	Colorless	Colorless	Colorless
Clarity	Clear	Clear	Clear
Stability	Up to 5 hours	Up to 12 hours	Up to 12 hours
Dilution with HCL 0.1N (1ml to 100ml)			
Color	Colorless	Colorless	Colorless
Clarity	Clear	Clear	Clear
Stability	Up to 5 hours	Up to 12 hours	Up to 12 hours
Dil. with Phosphate Buffer 6.8 N (1ml to 100ml)			
Color	Colorless	Colorless	Colorless
Clarity	Clear	Clear	Clear
Stability	Up to 5 hours	Up to 12 hours	Up to 12 hours

The dilution study reflect the behavior of prepared formulation as if it is taken orally and facing the fluids during Digestion system, firstly the stomach acidic environment, and then to Intestine basic environment.

The stability of the formulation during the dilution and for a period exceeding 12 hours indicate that the drug is stable within the period in which the drug stayed at stomach or Intestine. And these issue are critical steps determine the efficiency of the drug.

5.2.10. Stability Results:

Chemical stability:

There is no significant change in the active ingredient on the samples stored at room temperature, at 5 ± 3C and at 40 ± 2C, 75% RH in all samples for the mentioned study period and conditions.

Physical properties:

There is no significant change in the color, odor, pH, and appearance on the samples stored at room temperature, at 5 ± 3C and at 40 ± 2C, 75% RH for the mentioned study period and condition.

The study show that Atorvastatin microemulsion preparation is stable during the study period, no defects were observed during the mentioned study period with the ambient or

stress conditions, the pH stability indicate that the preparation still be stable and compatible when taken orally and will not decompose by the effects of low acidic pH. Atorvastatin calcium as an active pharmaceutical ingredient has a very low solubility at acidic pH or phosphate buffer, but the prepared Atorvastatin microemulsion can freely soluble in acidic pH of 0.1N HCl, phosphate buffer and water, above that the stability of the dilution assure that taken dosage will stay safe and could reach the Intestine to be absorbed effectively. The dosage of Atorvastatin microemulsion may have effectiveness more than ordinary labeled dosage of marketed oral tablet, which may help in determine the low dose of Atrovastain to be less than 10mg.

5.3. Applications in cosmetics:

For cosmetics applications the prepared system used to enhance the stability and solubility of Hydroquinone to develop a hydroquinone gel for Topical uses.

5.3.1. Preformulation:

A series of formulations were prepared using Lime oil as a solvent, Poloxamer 188 as a main surfactant and propylene glycol as co-solvent. The main dosage form prepared consisting of solutions and gel for topical application.

The formulation prepared listed in table (5.7):

Table (5.7): Composition for various formulation containing Hydroquinone.

Component	Qty in grams for 100 gram of microemulsion			
	F15	F16	F17	F18
Hydroquinone	1.900	2.1	2.10	2.10
Lime Oil	6.375	6.375	6.25	12.5
Propylene glycol	6.375	6.375	6.25	12.5
Poloxamer 188	56.00	56.00	37.5	25.0
Sodium metabisulfite	0.5000	0.5000	0.500	0.500
BHT	0.1000	0.1000	0.100	0.100
Water	31.25	31.25	50.0	50.0

Table (5.7) illustrated the main formulations preprared for hydroquinone gel and solution, numbers as F15, F16, F17 and F18, all the prepared samples prepared simultaneously at the same conditions of temperature and humidity and constructed for further investigations.

5.3.2. Specification of Finished Product:

The specifications and zero- time result for samples at room temperature illustrated in the table below:

Table (5.8): Results at Time of preparation (Zero Time).

Test	Limit	F15	F16	F17	F18
Color	Colorless	Colorless	Colorless	Colorless	Colorless
Odor	Lime fragrance	Lime	Lime	Lime	Lime
Appearance	Soft semisolid	Conform	Conform	Conform	Conform
Clarity	Clear	Clear	Clear	Clear	Clear
Viscosity	NMT 20000 cps	16000	15000	8000	6000
Date		25\10\12	25\10\12	25\10\12	25\10\12

NMT: Not More Than.

Table (5.8) describe the specifications of the prepared formulations numbered as F15, F16, F17 and F18, and indicate that there is no significant change within the prepared formulations.

5.3.3. Stability Result at Ambient Condition:

The prepared samples stored at room temperature for 1 month, the results illustrated on table (5.9):

Table (5.9): Stability Results at Ambient Conditions (Room Temperature) for 1 month.

Test	Limit	F15	F16	F17	F18
Color	Colorless	Colorless	Colorless	Colorless	Colorless
Odor	Lime fragrance	Lime	Lime	Lime	Lime
Appearance	Soft semisolid	Conform	Conform	Conform	Conform
Clarity	Clear	Clear	Clear	Clear	Clear
Viscosity	NMT 20000 cps	16500	16400	8200	5800
Date		25\11\2012	25\11\2012	25\11\2012	25\11\2012

Table (5.9) indicated that there is no significant change within the properties of the samples stored at room temperature for 1 month.

5.3.4. Accelerated Stability Study:

The prepared samples stored at 40 ± 2 C for 14 days, the results are in tables(5.10):

Table (5.10): Stability result at Accelerated Conditions (40 ± 2 C, 75% RH) for 14 days.

Test	Limit	F15	F16	F17	F18
Color	Colorless	Colorless	Colorless	Colorless	Colorless
Odor	Lime fragrance	Lime	Lime	Lime	Lime
Appearance	Soft semisolid	Conform	Conform	Conform	Conform
Clarity	Clear	Clear	Clear	Clear	Clear
Viscosity	NMT 20000 cps	15000	15000	7500	6000
Date		6\11\2012	6\11\2012	6\11\2012	6\11\2012

Table (5.10) indicated that there is no significant change within the properties of the samples stored at accelerated conditions for 14 days.

5.3.5. Stability at Refrigerator 5 ± 3C:

The results of the samples stored at temperature range (2 – 8)C for illustrated in table (5.11)

Table (5.11): Stability result at Refrigerator 5 ± 3 C for 1 month.

Test	Limit	F15	F16	F17	F18
Color	Colorless	Colorless	Colorless	Colorless	Colorless
Odor	Lime fragrance	Lime	Lime	Lime	Lime
Appearance	Soft semisolid	Conform	Conform	Conform	Conform
Clarity	Clear	Clear	Clear	Clear	Clear
Viscosity	NMT 20000 cps	10000	12000	5000	5000
Date		25\11\2012	25\11\2012	25\11\2012	25\11\2012

Table (5.11) indicated that there is no significant change within the properties of the samples stored at refrigerator for 1 month.

5.3.6. pH Test:

The range of pH for both gel and solution of preparations was (6.5 – 7.0)
All the prepared samples tested at zero time and at the end of study period was not exceeding the limit of pH at all storage conditions.

5.3.7. Stability Results:

Physical properties:

There is no significant change in the color, odor, appearance, and pH on the samples stored at room temperature, at 5 ± 3C and at 40 ± 2C, 75% RH for the mentioned study period.

There is no significant change in the samples during the mentioned study period which indicates that an easily prepared , effective, stable, compatible and safe of Hydroquinone

topical preparations are stable for at least 6 months and that is in accordance with approved guidelines for cosmetics registration on Palestinian ministry of health based on International Regulations.

Chapter Six
Conclusion

Chapter six
6.1 Conclusion

Poloxamer 188 triblock copolymer has a superior properties make it a promising material in different field of application, this research supplied unique results about poloxamer 188 tri-block copolymer.

The isotropic regions obtained from phase behavior of poloxamer 188 with lime oil indicate the main guidelines in spreading the study to investigate the effect of co-solvent, other nonionic surfactants and temperature on the poloxamer behavior.

Poloxamer 188 has 80% ethylene oxide, this unique properties make it more hydrophilic, and that noticed by the behavior of poloxamer toward preferable water solubility.

Addition Propylene glycol was helpful. It works as a surfactant and help in spreading the one isotropic region on the phase diagram, as discussed before propylene glycol molecule exist between hydrophilic block and hydrophobic blocks of the copolymer this effect make it work as a small surfactant beside tri-block copolymer.

The behavior of poloxamer 188 with Tween 80 was remarkable the isotropic one phase region seems promising to many field of industry, pharmacy, cosmetics and food, because the compatibility and safety of Tween 80, but Tween 80 make vigorous emulsion when applied to atorvastatin calcium microemulsion which reflected as a slightly turbid bluish emulsions, which finally conclude to separation of the preparation, this results lead to looking for other compatible nonionic surfactant. The Cremophore RH40 was the desired one.

The phase behavior of addition of cremophore RH40 to main system of poloxamer 188, Lime oil and propylene glycol gives a promising results and a formulation section of superior various preparations.

The sensitivity of poloxamer as nonionic surfactant appears clearly on the phase diagram of poloxamer 188 and cremophore RH 40 as surfactant, Lime oil and propylene glycol as oil phase, there is a clear shift and spreading to the isotropic one phase regions to oil rich corner. And the obtained phase diagram supply the research a strength and reliable results especially in pharmacy. Temperature of 37C is the body temperature it is much helpful to know the changes that may occur for the prepared formulation when applied as oral dosage forms.

Atrovastatin active ingredient is very important drug as lipid lowing agent, it used worldwide by differing age and dosage, the lowest dose supplied as oral tablet of 10mg, there is no known dose of atorvastatin microemulsion till the time of this research, the supplied research supply stable and applicable microemulsion formulation of 5 and 7.5 mg of atorvastatin calcium per ml of preparation.

Hydroquinone active ingredient as bleaching and anti-aging agent supplies as creams, gel, and solution for topical use, it supplied up to 5% in pharmaceutical products and up to 2% as OTC products the main issue is the stability of the product.

The research supplies a stable formulation of 2% hydroquinone gel for topical preparation. The unique properties of the hydroquinone gel that each component has different and important mission, relating to oil phase, Lime oil is superior components in facial products specially sunscreen and as perfuming material of the gel, the other component propylene glycol forming as a co-solvent and preservative agent material even its use in higher percentage in topical preparation for this purpose, there is a great benefit for using propylene glycol related to its biocompatibility, it works as penetration enhancer on skin. Hydroquinone gel for topical use could be treated as a brand formulation in cosmetics branch for its new component, stable, safe and compatible properties. Even in the research there is stable formulation for hydroquinone solution.

The research of formulation of hydroquinone has been restricted for only one system of the main studied system, and that is a great benefit in formulation. It's important to perform a product with minimum cost especially when the research treated as industrial scale, it is much better to perform a stable, effective, safe, high quality and low cost product than consuming a lot of component and introducing an extra costing manufacturing steps or equipment as heating, cooling.

This research provide unique properties of formulation depending on tri-block copolymer behavior, each phase diagram could be treated as a main guideline and as a map identify the prosperities and boundaries of each region, in which could be used as a safe selection for formulation.

6.2 Future Work

The research present a golden reliable result which can be used as a true real project and apply as Industrial production scale, work may farther investigated on Atorvsatatin microemulsion, investigation may done on using the preparations as soft gelatin capsule dosage form and clinical studies may accomplished, relating to hydroquinone topical gel a farther investigation may apply on sensitivity of skin and permeation of dug.

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Arabic abstract

المستحلب (**microemulsion**) يحضر من مكون مائي ومكون زيتي تربطهما مادة فعالة على السطوح تعرف (**Surfactant**), هناك عدة نظريات تتعلق بدراسة المستحلب من حيث التحضير والخصائص والثباتية, من أهم هذة النظريات ما يتعلق بأهمية وجود المادة الفعالة على السطوح ونسيتها, إذ أن الدرجة الأولى في الأهمية من استعمال المادة الفعالة على السطوح هو تخفيف التوتر السطحي (**Interfacial Tension**) بين مكونات المستحلب لتتم عملية الانسجام دون إضافات أو استهلاك للطاقة. من أهم مميزان المستحلب(**microemulsion**) الثباتية وسهولة التحضير وصغر حجم الجزيئات على المستوى المجهري الدقيق التي تبني التركيب الكلي فتعطيه الشكل الفيزيائي الشفاف والمتناهي في الصغر ويتم التعرف والكشف عن هذة الخصائص باستعمال أدوات متقدمة ومتطورة يمكن تبسيط فكرة عملها على اعتمادها على زاوية انعكاس الضوء عن المكونات الأساسية للأجزاء الدقيقة المكونة للمستحلب عند تعريضها لضوء بصفات وخصائص معينة.

إن الخصائص التي تميز المستحلب جعلته من أفضل وأوسع الأنظمة الحديثة التي يستعمل في كافة مناحي الصناعة والعلم خاصة الصناعات الدوائية والتجميلية والإلكترونية.
البوليمرات من أهم المواد الفعالة على السطوح التي تستخدم في تقليل التوتر السطحي لمكونات المستحلب, وتعرف البوليمرات على انها جزيئات ضخمة تتكون من لبنات بنائية تعرف الواحدة منها باسم مونمر وتترتب هذة المونمرات بطريقة معينة وبروابط بينية تعطي الشكل العام للبوليمر من حيث الحجم والكتلة الجزيئية والخصائص القطبية, واحيانا الاستعمالات التي يدخل فيها.

تركز الدراسة على الخصائص الدقيقة لبوليمر يعرف باسم بوليمر ثلاثي البنية, عبارة عن وحدة بناء ثلاثية التركيب وتتكون من نواه طبيعتها غير قطبية من مادو بوبلين اوكسيد وطرفين محيطين بالنواة ذات خصائص قطبية من مادة ايثلين اوكسيد. وتعرف بعدة انواع من حيث الخصائص الكيميائية والفيزيائية ومتاحة في السوق لكافة الاستخدامات الصناعية والطبية والتجميلية والالكترونية والجراحية.

يعود التميز في خصائص هذة البوليمرات الى طبيعة تكوينها من اللبنات الاساسية حيث تعطيها خصائص وتكون طبقات على المستوى المجهري الدقيق تظهر بشكل فيزيائي متنوع من هذة الطبقات تكوين جل شفاف وصلب وطبقات اخرى تتروح في اللزوجة والشفافية وطبعا في الخصائص الكيميائية اذ ان لهذة البوليمرات الثلاثية القدرة على الذوبان وتكوين تنوع في التركيب في كافة انواع المحاليل القطبية وغير القطبية. ويتم دراسة المادة الفعالة على السطوح وفعاليتها في تكوين طبقات **Microstructure phases** () باستخدام دراسة **(phase diagram).**

تركز الدراسة على استخدام نوع معين من المواد الفعالة على السطوح من البوليمرات الثلاثية يعرف باسم بوليكسيمير 188 , حيث تم دراسته وتحديد الخصائص والصفات لكل طبقة (**phase**) تم الحصول عليها في **phase)** **(diagram**, بالمراقبة الدقيقة لاي تغيرات تظهر خلال تحضير العينات وفحص العينات تحت الضوء (**(Polarized light**. وتم دراسة تاثير اضافة مواد مساعدة في تخفيف التوتر السطحي مثل مواد **Tween 80, Cremophore RH40** وكذلك مواد تساعد على الذوبان مثل مادة البروبلين جلايكول في أنظمة منفصلة وكذلك اختبار تأثير الحرارة على تصرف مادة البولكسمير**.**

الأهداف التي تم العمل عليها في الدراسة استخدام صفات وخصائص البولكسمير مع المواد الأخرى المساعدة لتحسين فاعلية دواء الاتروفاستيتن والهيدروكينون.
الاتورفاستيتين دواء يؤخذ لضبط مستوى الكوليسترول في الدم وهو من أكثر الأدوية المستعملة للكوليسترول في العالم ويؤخذ بجرعات دوائية بشكل حبوب صلبة تركيز المادة الفعالة فيها 10ملغم, 20 ملغم, 40ملغم و 80 ملغم. المشكلة الأساسية التي تواجه المريض في هذا الدواء ان نسبة الدواء التي يتم امتصصها في الجسم قليلة مقارنة مع الكمية التي تؤخذ بالجرعة ونسبة قليلة جدا تصل للدم. لذلك تم العمل على تحضير مستحلب يعتمد بشكل أساسي على البولكسمير كمادة فعالة على السطوح ومادة **Cremophor RH40** كمادة مساعدة ومادة زيت الليمون **Lime Oil** كمادة مذيبة ومادة بروبلين جلايكول كمادة مساعدة والماء كمذيب أساسي.
تم تحضير عينات بعدة تراكيز من المواد المذكورة وتحوي اكثر من تركيز من المادة الفعالة, وتمت دراستها على ظروف حرارة ورطوبة مختلفة تتراوح بين درجة حرارة الثلاجة 2-8 م ودرجة حرارة الغرفة وكذلك على 40 ±2 م ورطوبة نسبية 75%, لفترة تتراوح من شهر حتى 3 شهور وكانت النتائج ايجابية لأكثر من تركيبة.

المادة الدوائية الأخرى التي تم العمل عليها مادة الهيدروكينون, يستعمل الهيدروكينون كمادة مبيضة بالدرجة الالولى وبتراكيز لا تتعدى 5% في المجال الطبي وتراكيز لا تتعدى 2% في المجال التجميلي ويعرف في السوف باشكال جل وكريم ومحلول للاستعمال الخارجي على الجلد.

المشكلة الاساسية التي تواجه العمل على هذا المستحضر أن ثباتيته حساسة جدا ويعمل بحذر خاصة من الضوء , تم العمل في الدراسة على تحضير عينات من مستحضر الهيدروكينون بتراكيز تصل 2% و للاستعمال التجميلي, وتتكون من مواد البولكسمير والماء وزيت الليمون ومادة البروبلين جلايكول وتم دراسة الثابتية للعينات التي تم تحضيرها على ظروف حرارة ورطوبة تتراوح بين الثلاجة 2 – 8 م ودرجة حرارة الغرفة وحرارة 40 ±2 م ورطوبة نسبية 75%, لفترة تتراوح من 14 – 30 يوم وكانت النتائج جيدة جدا .