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Solubility and Stability of Natural Food Colorants in Microemulsions

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Löslichkeit und Stabilität natürlicher Lebensmittelfarbstoffe in Mikroemulsionen

Mohamed Awad Saad Abd El-Galeel

Es wurden verschiedene Mikroemulsionssysteme hergestellt unter Verwendung natürlicher Öle wie reines Pfefferminzöl und Mischungen mit anderen Speiseölen (Sojaöl, Erdnussöl, Rapsöl), verschiedenen Emulgatoren, wie Lecithin, Monoolein oder Tween20 und einer wässrigen Phase (Wasser oder eine 20%ige Lösung von NaCl, Saccharose oder Zitronensäure) ohne oder mit Ethanol als Co-Emulgator. Es wurde die Löslichkeit von Curcumin in diesen Mikroemulsionssystemen untersucht. Darüber hinaus wurde die Stabilität des in den Mikroemulsionen gelösten Curcumins gegen ultraviolettes Licht (UV) sowie gewöhnliches elektrisches Licht gemessen. Die Abnahme des Curcumin-Farbtönen wurde mithilfe eines Farbmessgerätes ($L^*a^*b^*$ -System) bestimmt und als Größe zur Bewertung der Stabilität herangezogen.

Die Ergebnisse zeigen, dass unter Verwendung geeigneter Komponenten Mikroemulsionen zur Verwendung in Lebensmitteln hergestellt werden können. Diese Mikroemulsionen erhöhen die Löslichkeit von Curcumin erheblich. Die Löslichkeit von Curcumin in diesen Mikroemulsionen ist besser als seine Löslichkeit in Ethanol, Speiseölen und Pfefferminzöl. Tween20/Pfefferminzöl-Mikroemulsionen zeigen die höchste Löslichkeit für Curcumin sowie die größte Wasseraufnahmekapazität. Alle in den Stabilitätstests vermessenen Mikroemulsionen lieferten gute Ergebnisse für die Stabilität von Curcumin gegen UV- und normales elektrisches Licht für einen langen Zeitraum, ausgenommen die Mikroemulsionen, deren wässrige Phase aus NaCl-Lösung bestand.

Die in dieser Arbeit hergestellten Mikroemulsionen scheinen vielversprechend für die Erhöhung der Löslichkeit und die Stabilisierung von Curcumin und anderer natürlicher Lebensmittelfarbstoffe mit vergleichbaren Eigenschaften (unlöslich in Wasser, schlecht löslich in Pflanzenölen und lichtempfindlich) zu sein.

Solubility and Stability of Natural Food Colorants in Microemulsions

Mohamed Awad Saad Abd El-Galeel

Several microemulsion systems were prepared by using different natural oils such as peppermint oil alone or mixed with a common edible oils (soybean, peanut or rapeseed oil), different surfactants such as lecithin, monoolein or Tween20 and an aqueous solution (water or 20% solution of NaCl, sucrose or citric acid) without or with ethanol as cosurfactant. The solubility of curcumin in these microemulsion systems was investigated. The stability of curcumin solubilized in these microemulsions against UV light and normal electric light was also studied. The stability of curcumin was determined as the change in curcumin color shade by using Measuring Color Instrument.

The results obtained indicated that microemulsions can be prepared by using a suitable components for food applications. These microemulsions greatly enhanced the solubility of curcumin. The solubility of curcumin in these microemulsions is more higher than that in ethanol, edible oils and peppermint oil. Tween20/peppermint oil microemulsions exhibited the highest solubility for curcumin and optimum water solubilization capacity. All microemulsions on the basis of the stability tests offered good results for curcumin stability against UV and normal electric light for long period, except microemulsions prepared by NaCl solution as aqueous phase.

These prepared microemulsions are suggested to be promising for solubility and stability of curcumin and other natural food colorants which have similar characteristics (insoluble in water, poorly soluble in vegetable oils and sensitive to light).

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1 Introduction

Microemulsions are generally defined as isotropic, transparent, thermodynamically stable mixtures of at least three components: a water, an oil and a surfactant; usually in combination with a cosurfactant, typically a short chain alcohol [Aboofazeli and Lawrence 1994]. Microemulsions are isotropic systems of infinite stability, where, the surfactant and the cosurfactant are principally located at the surface separating the two immiscible liquids to stabilize their mutual dispersion [Bourrel and Schechter 1988]. An interesting characteristic of microemulsions is that when even a small amount of a mixture of surfactant and cosurfactant is added to biphasic water-oil system, a thermodynamically stable, transparent and isotropic mixture spontaneously forms [Ho *et al.*, 1996].

When the components being used are safe for human consumption, microemulsions become important in such fields as foods, cosmetics and pharmaceuticals [Kunieda and Shinoda 1982]. Microstructural studies of microemulsions have been given great attention because of their physicochemical properties and various applications of commercial importance [Zaks and Klibanov, 1985]. Important in microemulsions is fundamental because of their presence in nature and applications in the food and pharmaceutical industries. A microemulsion is a stable and transparent solution of several components (usually oil, water and surfactant) with characteristic wavelength less than 100 nm to 200 nm [J. Research National Instit. Standards Technol. 1994].

The use of a microemulsion as reaction medium avoids the problem of insolubility frequently occurred with triglycerides and other lipophilic substrates. In addition, it opens new synthetic possibilities [Holmberg and Österberg 1987]. Recently, microemulsions have been identified as potential delivery systems for lipophilic agents due to their transparent or translucent appearance, stability for long time, high solubilization capacity and ease of formation [Malmsten 1996].

Since the first microemulsion system was described by Schulman and Hoar [1943] an extensive number of papers have been published in this field. Most of the systems published are, however, unacceptable for pharmaceutical use. A suitable oil phase for pharmaceutical uses would be vegetable oils [Von Corswant *et al.*, 1997]. Microemulsions prepared by using vegetable oils or fatty acid esters could be used, for example, in cosmetics and food products, where there is a demand towards environmentally more acceptable formulations

[Abillon et al., 1986]. Oils from natural sources and their derivatives, triglycerides, are considered to be harmless to the environment [Busch 1992], such systems with natural oils are but infrequently described in the literature [Alander and Warnheim 1989]. Few studies using triglycerides as the lipophilic phase in a microemulsion have been studied [Aboofazeli *et al.*, 1995]. The preparation of microemulsions with mineral oils, synthetic surfactants, and -if necessary- alkanols as cosurfactants has been intensively studied in the literature. Because, however, these components are harmful the problem is how to prepare microemulsions with nontoxic oils, surfactants and cosurfactants for possible applications in pharmaceutical industry [Kahlweit *et al.*, 1997]. The naturally occurring phospholipid (lecithin) are biocompatible and legislatively acceptable for food applications [Svensson *et al.*, 1996].

There are very few acceptable examples of ingestible microemulsions for food uses, even though much has been accomplished in recent years in the general field of microemulsions. microemulsions are suitable as the delivery system for water soluble and oil soluble nutrients, and flavors in foods. An ingestible, cosurfactant free system, with no off-taste or change in performance is suitable for this purpose [El-Nokaly *et al.*, 1991].

The advantages of a microemulsions over emulsions, or other solutions are either improved stability or solubility characteristics. Microemulsions also have the potential ability to solubilize both lipophilic and hydrophilic agents, which allows for a variety of flavoring and coloring materials having vastly different physical properties to be dissolved in the system [Friberg and Buraszenska 1978]. An additional important feature of phospholipid-based microemulsions is their ability to solubilize larger guest molecules [Peng and Luisi 1990]. From the above mentioned, the problem in the preparation of microemulsions generally is to find suitable components for food products.

Natural colorants are organic colorants that are obtained from natural edible sources using recognized food preparation methods, for example curcumin (from turmeric). Natural colors have always formed part of man's normal diet and have, therefore, been safely consumed for countless generations. The desirability of retaining the natural color of food is self-evident but almost the demands of industry are such that additional color is important. Contrary to many reports, natural sources can offer a comprehensive range of attractive colors for use in the food industry. Food quality is first judged on the basis of its color. The color of a food therefore influence not only the perception of flavor, but also that of attraction and quality. One of the advantages of using natural colors is that they are generally more widely accepted in food-stuffs than synthetic colors. It is only in the last 100 years or so that

synthetic colors have been added to food. For centuries prior to this, natural products in the form of spices, berries and herbs were used to develop the color and flavor of food. During this century, the use of synthetic color has gradually increased at the expense of these products of natural origin, due mainly to their availability and lower relative cost. In the last 20 years following the delisting of several synthetic colors there has been an increase in the use of colors derived from natural sources [Henry 1996].

The problem when using natural colors has been their lack of stability [Lauro 1998]. It should be remembered that natural colors are a diverse group of colorants with widely differing solubility and stability properties [Henry 1996]. Few plants have attracted the importance of scientists and been the subject of scientific studies. One from these plants has been investigated is *Curcuma longa* Linn [Cooper *et al.*, 1994]. Curcuma extracts have been shown to give a number of functions. It has been reported that administration of pure or commercial grade curcumin in the diet decreases the incidence of tumors in mice and also reduces tumour size. Histopathological test of the tumors showed that dietary curcumin inhibits the number of papillomas and squamous cell carcinomas of the forestomach as well as the number of adenomas and adenocarcinomas of the duodenum and colon [Azuine and Bhide 1994]. Turmeric has been used as a spice for many thousands of years. Curcumin is the principal color present in the rhizome of the turmeric plant. It is produced by crystallization from oleoresin, which is obtained by solvent extraction of the ground turmeric, and has a purity level of around 95% [Henry 1996].

It is important to note that the clinical trials of curcumin in human cancer patients are in progress [Ravindranath and Chandrasekhare 1980]. In addition, chemopreventive properties in skin and forestomach carcinogenesis and various pharmaceutical applications have been reported [Rao *et al.*, 1995]. Curcumin is an antioxidant that inhibits lipid peroxidation in rat liver microsomes, and a scavenger of reactive oxygen species that reduces the formation of inflammatory compounds such as prostaglandins and leukotrienes [Reddy and Lokesh 1992]. Curcumin has been extensively used to color and flavor in foods. Phenolic pigment is primarily caused by the yellow color of curcumin [Cooper *et al.*, 1994].

Curcumin is oil soluble but some blends of curcumin may be both oil and water insoluble. Curcumin needs the addition of gums, stabilizers or emulsifiers in order to render its miscibility in water. It is important that these ingredients are compatible with the food system to which the color is being required [Henry 1996]. Specification for curcumin [European Commission Document III/5218/94-rev.4, April 1995] states that the dye content must be not less than 90% when measured spectrophotometrically at 426 nm in ethanol, and

has ratio of curcumin to essential oil 99:1. Curcumin is not an ideal product for direct use in the food industry since it is insoluble in water and has poor solubility in most oils used in food products. Thus it is important for curcumin to be converted into convenient application form. In many countries, this is achieved by dissolving the curcumin in a mixture of food-grade solvent and accepted emulsifier. In this form, the product contains 4 to 10% curcumin and easily dissolves in water. Polysorbate 80 is the favoured emulsifier/diluent for such products since it is an ideal carrier for curcumin. Curcumin is sensitive to light and this factor is the one that generally limits its use in foods [Henry 1996].

The problems for curcumin usually are its solubility and stability against light, because it is insoluble in aqueous solutions and poorly soluble in fats or oils that are used in foods and it is sensitive to light. Microemulsions have infinite stability and large solubility capacity for lipophilic and hydrophilic substances as well as ability to solubilize larger guest molecules.

Microemulsions also were used successfully to solubilize and stabilize some pharmaceutical components such as cyclosporin A by Gao *et al.*, [1998] and to stabilize some nutrients such as ascorbic acid against oxidation by Gallarate *et al.*, [1999]. For these reasons, microemulsions were chosen to investigate the curcumin solubility in order to use it with food products and study the possibility of these microemulsions to protect the curcumin against light during the storage.

2 Theoretical Background

2.1 Characteristic of microemulsions

The most characteristic difference between an emulsion and a microemulsion is their appearance. An emulsion is turbid while the microemulsion almost is transparent. The reason for this difference in appearance is the size of the droplets. For an emulsion the droplets are similar or greater than the wave length of light and light is reflected off their droplets. The emulsion, hence, appears turbid because the light cannot penetrate through it. On the other hand the size of microemulsion droplets is smaller than the wave length of light, and the interaction with light is limited to scattering. The light beam passes through with but little loss; consequently the microemulsion appears transparent. The microemulsions are thermodynamically stable with few exceptions, but the emulsions are not thermodynamically stable because the interfacial energy is positive and dominant in total free energy, where its droplet is of a size that the bending energy is negligible and the surface free energy is large and positive; a few mN/m. The surface free energy of the microemulsion has two components stretching (positive contribution) and bending (negative contribution). The two cancel each other and the total surface free energy is extremely small about 10^{-3} mN/m (figure 1). Moreover, The microemulsions form spontaneously or need gentle shaken to miscible the components for short time (few minutes). The most characteristic differences between an emulsion and a microemulsion are presented in Table 1 [Friberg and Kayali 1991]. These microemulsions can take different forms which include among others oil water and oil/water bicontinuous microemulsions. Phase change can occur between these different forms due to changes in either individual component concentrations or other thermodynamic conditions [Rosen 1989].

Table 2.1. Characteristics of Emulsions and Microemulsions [Friberg and Kayali 1991]

	Emulsion	Microemulsion
Appearance	Turbid	Transparent
Droplet size, μm radius	0.15 – 100	0.0015 - 0.15
Formation	Mechanical or Chemical Energy	Spontaneous
Thermodynamic Stability	No	Yes (No)

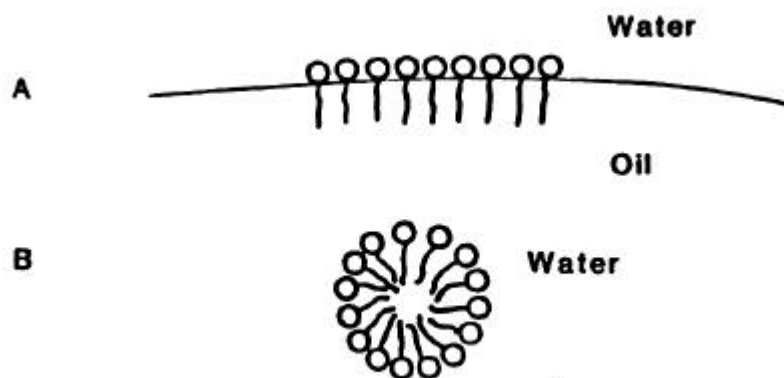


Figure 2.1. The curvature in an emulsion droplet (A) is extremely small and the bending component of surface energy is not significant. A change in curvature does not lead to a change in the free energy. In the microemulsion droplet (B), on the other hand, a change in curvature leads to a pronounced change in free energy; e.g., the bending component of the surface free energy is pronounced [Friberg and Kayali 1990].

The microemulsions possess special characteristics of relatively large interfacial area, ultra low interfacial tension and large solubility capacity as compared to many other colloidal systems [El-Nokaly *et al.*, 1991].

The surfactant to cosurfactant ratio greatly affected the physicochemical characteristics of the resultant microemulsion systems obtained using polyoxyethylated castor oil (Cremophor EL) as a surfactant, Transcutol as a cosurfactant and carylic/capric triglyceride (Captex 355) as an oil. The stable microemulsion with its high solubility of cyclosporin A (poorly water-soluble drug), small droplet size and fast dispersion rate was obtained from a mixture composed of 10:5:4 ratio of Cremophor EL:Transcutol:Captex 355. The enhanced bioavailability of cyclosporin A loaded in this microemulsion system might be attributed to the small droplet size of microemulsion systems [Gao *et al.*, 1998].

2.2 Structure and formation of microemulsions

Bansal *et al.*, [1980] reported that the conditions necessary for microemulsion formation are:

- large adsorption of surfactant or surfactant/cosurfactant mixture at the interface between the oil and water which achieved by choosing a surfactant mixture with proper hydrophilic-lipophilic-balances (HLB).
- high fluidity of the interface. The interfacial fluidity can be developed by using a proper cosurfactant or an optimum temperature.

- optimum curvature. The importance of oil penetration in the surfactant/cosurfactant film and the appropriate surfactant/cosurfactant structures.

The microemulsions can take different types which include, among others, oil, water and oil/water bicontinuous microemulsions. Phase change can occur between these different forms due to changes in nature or concentrations of individual component or other thermodynamic conditions [Rosen 1989].

A microemulsion that contains a relatively low content of oil confined within small isolated droplets dispersed in water is known as oil-in-water (O/W) microemulsion, while the reverse type (small amount of water dispersed in large amount of oil) is a water-in-oil (W/O) microemulsion. Upon continuously increasing the water-to-oil ratio in a W/O microemulsion, phase inversion occurs. An intermediate transparent, isotropic bicontinuous structure may form during such an inversion, involving both oil and water-continuous domains separated by interfacial surfactant film [Singh, *et al.*, 1994]. Under appropriate conditions the microemulsion system is miscible with both oil and aqueous phase. However, the microemulsion system partitions into three phases, a surfactant-rich phase, a surfactant-rich aqueous phase and a surfactant-rich oil phase. The surfactant-rich phase is called a middle phase microemulsion [Abe, *et al.*, 1986]. It is in the middle phase microemulsion where a surfactant shows the greatest solubilizing power for both water and oil; here it also gives ultra small values of interfacial tensions between oil and water which are less than 10^{-2} mNm⁻¹ under proper conditions [Kunieda and Shinoda 1982]. The nature and structure of the surfactant, cosurfactant and oil are important factors in the preparation of microemulsions [Ho, *et al.*, 1996]. The ability of phospholipids to form microemulsions with alkanes has been studied by several authors [Shinoda and Kaneko 1988, Shinoda *et al.*, 1991 and 1993, Schurtenberger *et al.*, 1993, and Kahlweit *et al.*, 1995]. Shinoda *et al.*, [1993] have shown that it is possible to form microemulsions with equal amounts of hexadecane and aqueous phase with only 2.5% soybean phospholipid using 1-propanol as the cosurfactant, and Kahlweit *et al.*, [1995] have systematically studied the influence of chain length of both the phospholipid and the hydrocarbon on the microemulsion phase behavior.

Aboofazeli *et al.*, [1994] studied partial phase diagrams of systems containing water, egg lecithin, propanol and different polar oils such as Miglyol 812 and soybean oil, and found that the influence of the oil and the ratio of egg lecithin to propanol on the microemulsion area is significant. Due to the salting-out effect, the addition of NaCl decreases the required amount of pentanediol (as cosurfactant), but has only a small effect on the lecithin required (as surfactant) [Kahlweit *et al.*, 1995].

2.2.1 Role of surfactant and cosurfactant:

Surfactant and cosurfactant are mainly located at the surface separating the two immiscible liquids (usually oil and water) to stabilize their mutual dispersion [Bourrel and Schechter 1988]. A hydrophilic surfactant adsorbs strongly to an interface toward the air or toward an oil because of its dual structure with a hydrocarbon tail with limited interaction with water (the hydrophobic part) and a polar group with strong interaction with water (the hydrophilic part). This adsorption acts a reduction of the interfacial free energy. An lipophilic surfactant does not adsorb toward the oil/air interface, but does so toward an oil/water interface. For surfactant concentrations above a certain limit in water (the critical micellization cocentration,c.m.c.) the added surfactant forms micelles, figure 2(A), and the adsorption to the interface does not increase with surfactant concentration. In an oil, the oil soluble surfactants and water form inverse micelles, figure (2B), in a step-wise process. These two structures are especially significant in systems in which an ionic surfactant and a long chain alcohol are combined, because this system illustrates, with high clarity, the fundamental difference between the stabilizing system for a microemulsion and an emulsion. Once this difference is distinguished the difficulties with microemulsions in food products easy to comprehend [Friberg and Kayali 1991]. A cosurfactant is almost a medium chain fatty alcohol, acid or amines [Lang *et al.*, 1984].

The role of the cosurfactant together with the surfactant is to reduce the interfacial tension down to a very small even transient negative value at which the interface would expand to form ultra small dispersed droplets, and consequently adsorb more surfactant and surfactant/cosurfactant until their bulk condition is depleted enough to give interfacial tension positive again. This process known as „spontaneous emulsification“ forms the microemulsion. Thus, based on ability of the cosurfactant to affect the solvent properties of oil and/or water and to penetrate the surfactant interfacial monolayer, **it can:**

- Reduce further the interfacial tension. Increase the fluidity of interfaces.
- Destroy liquid crystalline and/or gel structures which prevent the microemulsion formation.
- Adjust HLB value and spontaneous curvature of the interface by changing surfactant partitioning characteristics.
- Decrease the sensitivity to structure fluctuations and brings formulation to its optimum state [Kunieda *et al.*, 1988].

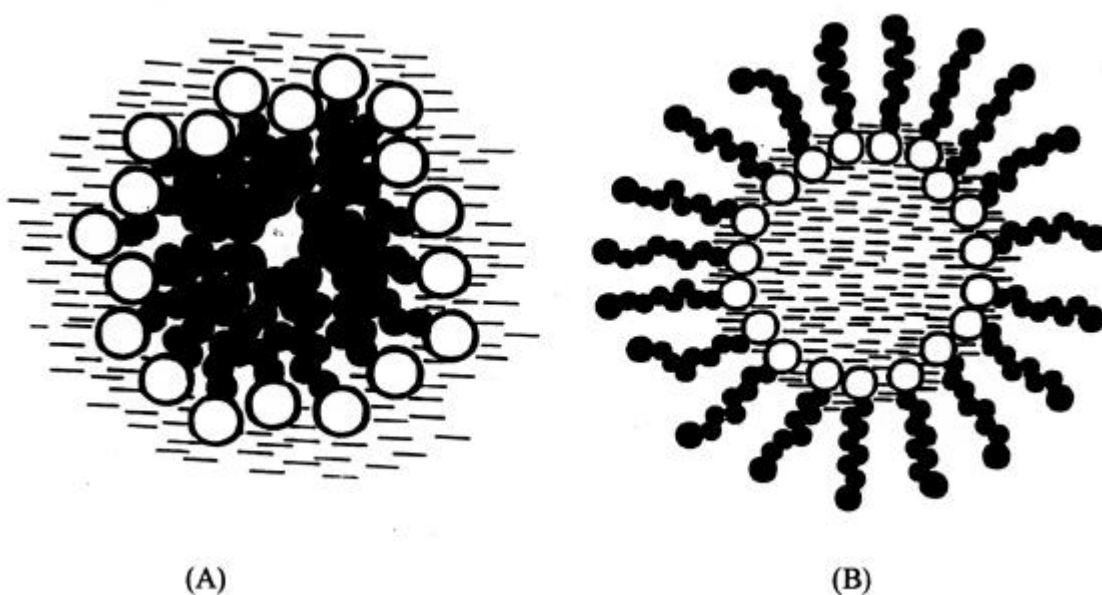


Figure 2.2.(A) normal micelle, in this case the surfactant hydrocarbon chains (black) point toward the inner part surrounded by the polar parts (unfilled circles).(B) inverse micelle in this case the hydrocarbon chains point outward while the polar groups are concentrated in the center [Friberg and Kayali 1991].

The nature and concentration of the surfactant become of too importance to obtain an optimum solubilization in a given W/O microemulsion [El-Nokaly *et al.*, 1991]. Polyoxyethylene sorbitan trioleate (Tween 85) is a nonionic surfactant which has some important properties for microemulsion preparation and protein solubilization [Komives *et al.*, 1994]. The cosurfactant can act by either interchelating between surfactant molecules at the interface between oil and water and/or by decreasing the aqueous phase hydrophilicity. In the preparation of a balanced lecithin microemulsion, the cosurfactant has an additional role in that it can also act to decrease the tendency of lecithin to form a highly rigid film [Binks *et al.*, 1989], thus allowing the interfacial film to take up the different curvatures required to prepare balanced microemulsions [De Gennes and Taupin, 1982]. Because alkanols are, in general, toxic, can substituted by alkanediols. The inexpensive alkanols can use in industry as cosolvents and their toxicity can be tolerated, whereas in pharmacy one may have to use nontoxic alkanediols [Kahlweit *et al.*, 1995].

2.2.2 Oils used in the microemulsion preparation

The preparation of microemulsions with mineral oils, synthetic surfactants, either ionic or nonionic, and -if necessary- alkanols as cosolvents, has become a well-established practice [Kahlweit 1995]. Because, however, mineral oils and synthetic amphiphiles, as well as alkanols, in general, harmful, the problem is how to prepare microemulsions with suitable components for possible applications in pharmaceutical industry [Kahlweit *et al.*, 1997]. Unfortunately, most work to date studying microemulsions has utilized oils, surfactants and cosurfactants unacceptable for pharmaceutical purposes. In order to make these systems pharmaceutically acceptable, it is necessary to prepare such systems by using nontoxic and safe components [Aboofazeli and Lawrence 1994].

Oils from natural sources and their derivatives, e.g. triglycerides and fatty acid methyl esters, are easily degraded by microorganisms and are considered to be harmless to the environment [Busch 1992]. The formation of bicontinuous microemulsions with mineral oils has been intensively investigated in model experiments [Kahlweit *et al.*, 1990] and for application in industrial products [Schwuger 1995], such systems with natural oils and esters are but infrequently published in the literature [Alander and Warnheim 1989].

An acceptable lipophilic phase for pharmaceutical uses would be a vegetable oils [Von Corswant *et al.*, 1997]. The extension of a microemulsion region is generally dependent on the oil nature. This is due to differences in oil penetration into the surfactant layer [Monduzzi *et al.*, 1997]. Relatively few studies using triglycerides as the lipophilic phase in a microemulsion have been studied [Aboofazeli *et al.*, 1995]. Joubran *et al.*, [1994] studied microemulsions of soybean oil, polyoxyethylene(40) sorbitan-hexaoleate, and water-ethanol. They found that the extension of the water-in-oil (W/O) microemulsion regions were significantly dependent on temperature. Moreover, water-ethanol ratios also affected the phase behavior. In these systems large amounts of the surfactant had to be used to form a microemulsion with equal amounts of triglyceride and aqueous phase.

Triglycerides form bicontinuous microemulsions only at relatively high temperatures with a high amount of hydrophobic surfactants owing to their high molecular weight. Esters of fatty acids, however, offer suitable phase behavior forming three phases at appropriate temperature and moderate surfactant content [Mönig *et al.*, 1996].

Alander and Warnheim [1989a] observed that microemulsion phase diagrams for high-molecular-weight triglycerides exhibit smaller homogeneous regions than low-molecular-weight esters and hydrocarbons. This effect has been attributed to better penetration of the interfacial film by small oil molecules which aids in obtaining optimal curvature of W/O or O/W droplets [Walde *et al.*, 1990].

Triglycerides, in particular large triglycerides such as peanut oil, are significantly more difficult to solubilize into microemulsions than hydrocarbons or alkyl esters [Alander and Warnheim 1989a].

El-Nokaly *et al.*, [1991] explained the reasons which make difficult concerning to form a triglyceride microemulsions:

- Triglycerides are semi-polar comparing with hydrocarbons.
- A surfactant of higher hydrophile-lipophile-balances (HLB) is thus needed to favor the water-in-oil system, with lower solubility in bulk and increased adsorption at the interface.
- In case of triglyceride, the ratio of surfactant/water is high.
- The surfactant efficiency is decreased if it lost to the bulk and is unavailable to the interface.

Edible triglycerides such as soybean, rapeseed, or sunflower oils contain long alkyl chains mainly C16, C18, C20 and C22. The oil may be very bulky to penetrate the interfacial layer to assist the formation of the optimum curvature, figure 2.3. Reports of oil being solubilized in the aggregates palisade layer may be due to the shortness of the alkyl chains in the triglycerides used [Kunieda *et al.*, 1988].

To increase the triglyceride microemulsion regions, a different strategy was applied by using a suitable hydrotrope to destabilize the liquid crystalline phase of the triglyceride, surfactant and water, which leads preferentially to the formation of the microemulsion [Joubran *et al.*, 1993]. The preparation of triglyceride microemulsions can be achieved by incorporating sucrose and short chain alcohols such as ethanol. The alcohol acts synergistically with sucrose to destabilize the liquid crystalline mesophase [Joubran *et al.*, 1994]. Sucrose enhanced the formation of the oil-in-water microemulsion phase while destroying the water-in-oil microemulsion phase. Triglycerides containing unsaturated or short-chain fatty acids have improved solubility in oil-in-water microemulsions compared to triglycerides with saturated or long-chain fatty acids [Parris *et al.*, 1994].

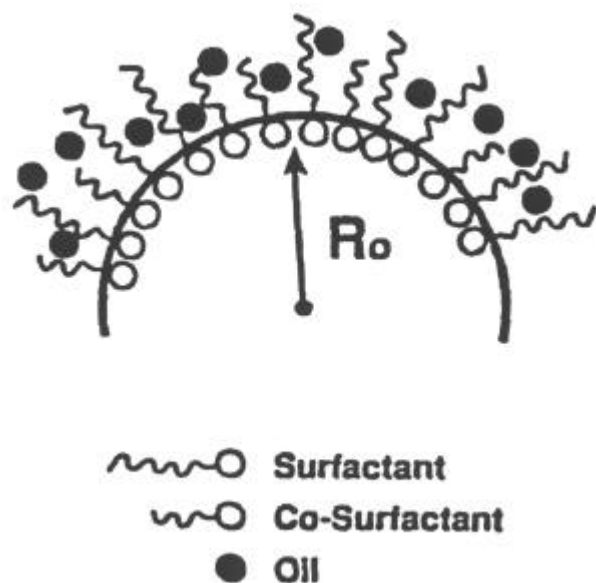


Figure 2.3. Optimum Curvature, R_o =Radius of Spontaneous Curvatures [Kunieda *et al.*, 1988].

2.3 Applications of microemulsions

As a consequence of their unusual thermodynamic properties, microemulsions are of considerable industrial importance in tertiary oil recovery [Langvin 1984], extraction of biomolecules from fermentation broths [Göklen and Hatton 1985] and as liquid membrane carrier agents [Tonder and Xenakis 1982]. W/O microemulsions with little water content exhibit the best cleaning results for oil soil [Dörfler *et al.*, 1995]. So far microemulsions have not been exploited for pharmaceutical purposes [Aboofazeli and Lawrence 1994].

In recent years, microemulsions have been identified as potential drug delivery systems for lipophilic drugs due to their transparent or translucent appearance, long term stability, high solubilization capacity, and ease of preparation [Malmsten 1996]. Water-in-oil (W/O) microemulsions are a particularly attractive system for biotechnological applications [Stamatis *et al.*, 1995]. Microemulsions of edible oils in a matrix of water and different hydrotropes have been used as carriers for flavors or essential oils [Wolf and Hauakotta 1989].

Since alcohols of medium chain length (generally used as cosurfactant) tend to possess unacceptable toxicity/irritation profiles (see Table 2.2, their use in foods is very limited. Unlike, the ethanol ingestion is of known consequence. Thus in small amounts (5%), use of ethanol is completely acceptable for food products [Osborne *et al.*, 1991].

Table 2.2 Safety considerations of Medium Chain Alcohols [Osborne *et al.*, 1991]

Alcohol	Oral LD50 in Rat (mg/kg)	Eye Irritation	Skin Irritation
2-Butanol (least irritation of 4 isomers)	6480	Moderate	Threshold Conc. 7.8%
Hexanol	720	Severe	Mild
Octanol	1790	Moderate	Threshold Conc. 12%
Decanol	472	Sereve	Sereve

Oral LD50 for mouse, oral LD50 for humans 0.5-5 mg/kg

Microemulsions have the potential ability to solubilize both lipophilic and hydrophilic species, which allows for a variety of flavoring and coloring agents having vastly different physical properties to be dissolved/solubilized within a system. A major disadvantage of microemulsion systems for ingestion is the traditional need for a medium chain alcohol such as pentanol to function as a cosurfactant [Friberg and Burasczenska 1978].

Microstructural studies of microemulsions have been given considerable attention because of their interesting physicochemical properties and various applications of commercial importance [Zaks and Klibanov 1985]. Interest in microemulsions is substantial because of their ubiquitous presence in nature and applications in the food and pharmaceutical industries. Further studies are under way with the goal of aiding development of improved properties and stability of microemulsions used in many processed food products [J. Research National Instit. Standards Technol. 1994].

Microemulsions containing vegetable oils or fatty acid esters could be used, for example, in cosmetics and food products, where there is a demand for environmentally more acceptable formulations [Abillon *et al.*, 1986]. Phospholipid-based (W/O) microemulsions have ability to solubilize larger guest molecules such as enzymes [Peng and Luisi 1990]. It has been shown earlier that lipases in microemulsions can be successfully used to catalyze esterification reactions [Kolisis *et al.*, 1990]. When ionic surfactants are used, the resulting microemulsions are often called reverse micelles [Hatton 1989].

Many enzymatic reactions require biphasic media, polar media for the solubilization of enzymes, and organic media solubilization substrates. Reverse micelles provide a larger polar/apolar interfacial area, hence improving the interaction between enzyme and substrate [Hayes and Gulari 1990]. Use of reverse micelles have many advantages including easier media and enzyme preparation, less mass-transfer limitations (due in part to its large degree of interfacial area), and simpler control and monitoring of water content [Hayes and Gulari 1991]. The use of a microemulsion as reaction medium eliminates the problem of insolubility frequently encountered with triglycerides and other lipophilic substrates. In addition, it opens novel synthetic possibilities. For instance, lipase catalyzed interesterification can be used to produce triglycerides, which is interest for the production of synthetic cocoa butter [Holmberg and Österberg 1987].

From an industrial point of view, the microemulsions could be attractive systems for making stable products with low fat content [Friberg *et al.*, 1990]. Monoglycerides are nonionic surfactants widely used as emulsifiers in the food and pharmaceutical areas. Normally they are produced by alcoholysis of corresponding triglyceride with two equivalents of glycerol. The reaction requires high temperatures (210-240 °C) and the use of a transesterification catalyst. After work-up the effective yield of triglyceride to monoglyceride conversion is 40-50%. Monoglycerides have been obtained in 80% yield by enzyme catalyzed hydrolysis of the corresponding triglyceride. The reaction was carried out in an oil-rich microemulsion formulated without cosurfactant [Holmberg and Österberg 1988]. The ability of W/O microemulsion to isolate and selectively extract proteins is well-known [Pires *et al.*, 1996]. The use of nonionic surfactant microemulsions for protein extraction has attracted attention recently [Vasudevan *et al.*, 1995].

2.4 Food microemulsions

The food emulsions have always generated great interest. However, little has been done with microemulsions applicable to the complex world of foods. Microemulsions have been the subject of much fundamental research that focuses on noningestible systems. For example, applications to non-food uses such as tertiary oil recovery, fuel, cosmetics, and household have received considerable attention. Larsson's monoglyceride/water/oil system was the first practical system to be published [El-Nokaly *et al.*, 1991].

Since the first microemulsion system was described by Schulman and Hoar [1943] an extensive number of papers have been published in this area. Most of the systems described are, however, not suitable for pharmaceutical use [Von Corswant *et al.*, 1997].

The yield from this search (Food micremulsion) was a mere four references none older than 1987. The inclusion of microemulsions is a matter of looking to the future [Becher 1991]. It should point out that a recent review of applications of micremulsions contained but a single reference to foods [Gillberg 1984]. In fact, a number of recent books on food emulsions, edited by; respectively, Friberg 1976, Dickinson 1987, and Dickinson and Stainsby, 1988, contain no instance of food microemulsions (except for one minor reference in Dickinson 1987). This lack of interest may possibly be ascribed to a number of related factors. First, the high levels of emulsifying agents normally encountered in microemulsions quite simply serves as an economic barrier. Second, this same high level of emulsifier might well raise legal problems in securing approval from the FDA. Third, of course, there is the simple fact that it is apparently quite difficult to make microemulsions of the fats and oils used in foods [Becher 1991]. From these considerations, food microemulsions, it follows automatically that a surfactant/cosurfactant combination, which is optimal for a microemulsion, is of little use in order to stabilize an emulsion. This is a serious disadvantage when double emulsions, W/O/W, are formulated. For a system of this kind a W/O microemulsion emulsified into water would in principle be a very attractive option, because the W/O part which is the difficult part to stabilize would now be thermodynamically stable. However, stabilization using surfactant combination has fundamental difficulties. The surfactant combination for the microemulsion will rapidly exchange with the one for the emulsion, which leads to destabilization for both the emulsion and the microemulsion. This dilemma has been resolved in an elegant manner by Larsson *et al.*, [1980]. They used a surfactant to stabilize the W/O microemulsion but avoided the problem of emulsion part by using a polymer as its stabilizing agent. The polymer, being water soluble, is virtually insoluble in the oil part of the microemulsion and its dimensions prevents its inclusion into W/O droplets. It will, hence, not interfere with the W/O microemulsion stabilization system.

As pointed out earlier liquid triglycerides do not lend themselves to microemulsion formulation with the traditional technique. Addition of liquid triglycerides to the inverse micellar solution results in a phase change to a lamellar liquid crystal. Hence, a different strategy must be employed in order to prepare a microemulsion with triglycerides. One solution is to attack the problem from the opposite side. Realizing that the microemulsions are obtained by destabilizing a liquid crystal, figure 2.4, it appears reasonable to approach the problem with a liquid crystal as starting point instead. This means forming a liquid crystal containing triglycerides and destabilizing it by addition of a suitable compound. This compound should be considered as a potent cosurfactant destabilizing the liquid crystalline

phase. The common cosurfactants are, however, not useful because of their toxicity. The destabilization was instead obtained by the use of hydrotropes [Friberg and Rydhag 1971]. These are compounds, the action of which is the destabilization of liquid crystals as was early demonstrated [Lawrence and Pearson 1964]. A large number of them are allowed into food products. The solution in the system of water/1-monocaprylin/sodium xylene sulfonate is the largest one and it was used to dissolve a triglyceride, trioctanoin. The amount of triglyceride dissolved was very indeed with a maximum of 13.5% by weight of triglyceride. Other approaches have resulted in similar results. So, for example, does the system water/monocaprylin/tricaprylin which was prepared by Ekwall [1975], shows very little water solubilization into the oil. To reach 15% by weight of water, 50% of the stabilizers were needed [Friberg and Kayali 1991].

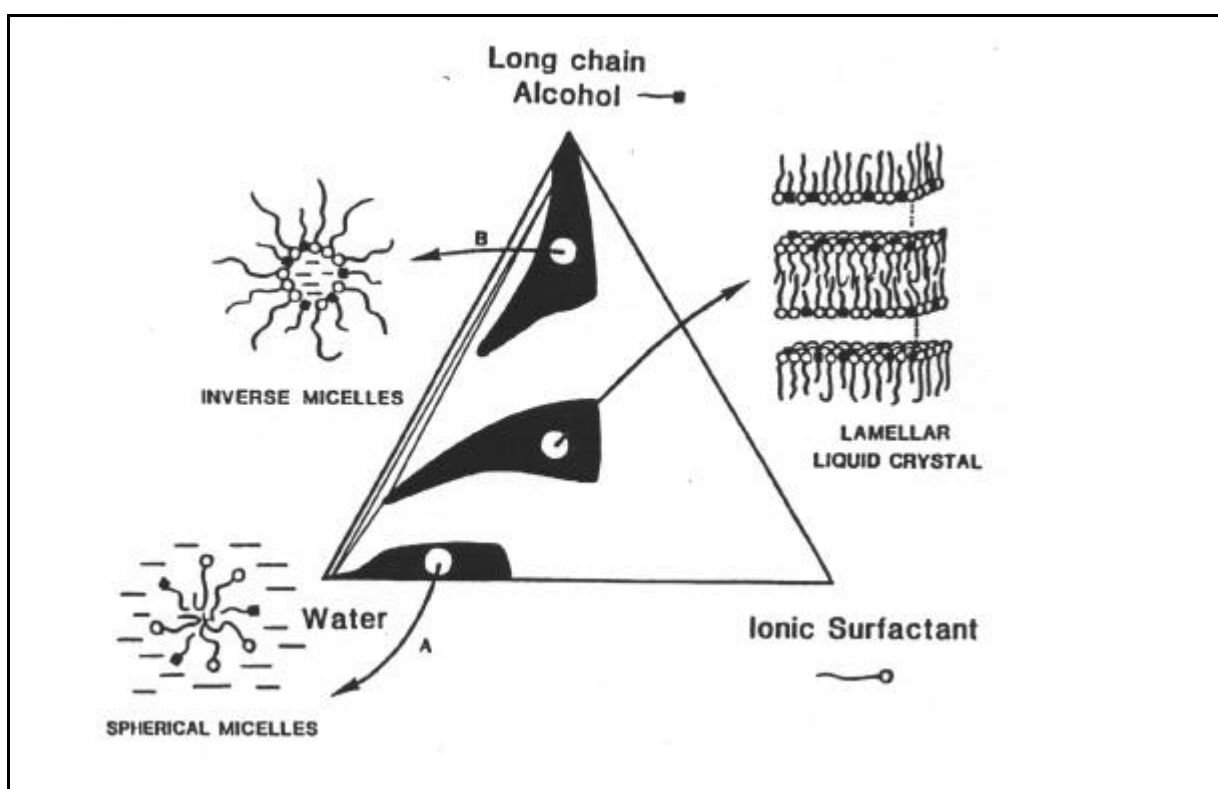


Figure 2.4 In a combination of water, a surfactant and a long chain alcohol, the areas for solutions of normal and inverse micelles, are separated by a lamellar liquid crystal [Friberg and Rydhag 1971].

There are very few available examples of ingestible water-in-oil or oil-in-water microemulsion systems for food products, even though much has been accomplished in recent years in the general field of microemulsions. A water-in-oil (W/O) microemulsion is suitable as the delivery system for water soluble nutrients and flavors in foods. An ingestible, cosurfactant free system, with no off-taste or change in performance (soybean oil, water and commercially available food surfactants such as polyglycerol oleate, polyglycerol linoleate monoglyceride and polyoxyethylene sorbitol oleate) was chosen for this purpose. The surfactants are evaluated based on their structures and performance in solubilizing water in high triglyceride concentration range [El-Nokaly *et al.*, 1991].

Microemulsions prepared by using triglycerides and other food components, polyoxyethylene(40) sorbitol hexaoleate, anionic surfactant, were studied by Joubran *et al.*, [1993], and soybean oil was used as a typical triglyceride.

2.4.1 Earlier work on food microemulsions applications:

Reports on milk fortified with vitamin A solubilized in a microemulsion have been published [Duxbury 1988]. Dissolving essential oils in water with and without an alcohol cosurfactant for aromatization of beverage or pharmaceutical formulations, have been reported [Wolf and Havekotte 1989]. A new system for hydrolyzing milk fat was developed by using a microemulsion system containing reversed micelles [Chen and Pai 1991]. Controlled hydrolysis of milk fat by lipase is applied in the dairy industry to produce lipolyzed milk fat with butter-like or cheese-like flavor [Kilara 1985]. The microemulsion single phase region of a peppermint oil/Tween20/water system which was studied by Treptow [1971]. Friberg and Rydhag [1971] solubilized up to 15 wt% tricaprylin in an isotropic aqueous solution of monocaprylin and a hydrotrope such as sodium xylene sulfonate. Treptow [1971] dissolved less than 10% soybean oil in water with a 30/70 surfactant mixture of Tween 20 and G1045 respectively. Ascorbic acid was added to the emulsified systems, such as microemulsions, and its stability against oxidation was studied at 45 °C in aerobic conditions and compared with that in aqueous solutions at different pH values. All emulsified systems provided protection to ascorbic acid, as its degradation rate, was slower in emulsified systems than in aqueous solutions [Gallarate *et al.*, 1999].

The rate of oxidation of linolic acid and ethyl linoleate in O/W microemulsions was studied by Carlotti *et al.*, [1995]. The results showed that the structure of the interface in the microemulsions was particularly important to protect the systems from auto-oxidation.

An alcohol or acid cosurfactant was needed to form the microemulsion, with the exception of the work done by Gulik and Larsson [1984] and Troptow [1971]. Such cosurfactants are usually not acceptable for taste, safety, or performance reasons in ingestible oil formulations. The scarcity of work available on water solubilization in triglyceride for food applications is obviously due to difficulties inherent in the structure of oil, in finding appropriate cosurfactants, and the need to use minimum amounts of food approved surfactants not to adversely affect the oil properties [El-Nokaly *et al.*, 1991].

2.4.2 Surfactants in food microemulsions

When the surfactant being used is safe for human consumption, microemulsion become important in such fields as foods, cosmetics and pharmaceuticals [Kunieda and Shinoda 1982]. Surfactants in food are usually called emulsifiers whether their intended use is emulsification or not. An Acceptable Daily Intake (ADI) value has been allocated to most food emulsifiers by health authorities in many countries (FDA, FAO, EEC) [El-Nokaly *et al.*, 1991]. Chemically, most food surfactants are esters of fatty acids with naturally occurring alcohols and acids. The primary raw materials for food surfactants production are fats and oils which can be utilized directly or after having been hydrogenated, fractionated, or split to fatty acids and glycerol [Lauridsen 1986], they have to impart no taste or smell on foods. They are mostly nonionic surfactants with few exceptions such as succinic, citric, and diacetyl tartaric acids esters of monoglycerides and soaps. Amphoteric lecithin is the only food approved surfactant containing a positive charge. Surfactants listed in Table 2.3, were tested for W/O microemulsion formation at 12:1 surfactant/water by weight. The oleate and linoleate groups were preferred for testing because of their unsaturation and its known effect in increasing fluidity of interfacial film. The sorbitan and sorbitol esters of oleate and linoleate did not give microemulsions at surfactant/water ratio below 12:1. Branching should be another factor in increasing the interfacial fluidity. The special kink in the linoleate molecule which makes its alignment difficult, thus leading to an increase in the interfacial fluidity. The presence of unsaturation was found to be important. The monooleate and linoleate could act as cosurfactant since they are different in hydrocarbon moiety size comparing with dilinoleate [El-Nokaly *et al.*, 1991]. For legislative reasons synthetic surfactants are in any case of limited use in food synthesis applications. A group of surfactants which possess no such limitations are the naturally occurring phospholipid (lecithin) surfactants which are biocompatible and legislatively acceptable [Svensson *et al.*, 1996].

Table 2.3 Chemical Classification of Food Emulsifiers and Legal Status (US FDA 21 CFR*)

General Class	Example	ADI Values** mg/kg body wt/day
Partial Glycerides	Mono- and diglycerides	Not limited
	Lactic acid esters of monoglycerides	Not limited
	Acetic acid esters of monoglycerides	Not limited
	Citric acid esters of monoglycerides	Not limited
	Diacetyl tartaric acid esters of monoglycerides	50
	Succinic acid esters of monoglycerides	3% by wt. of shortening; 5% by wt. of flour
Fatty Acid Esters of Polyols	Propylene glycol esters of fatty acids	25
	Polyglycerol esters of fatty acids	25
	Sucrose esters of fatty acids	10
	Sorbitan esters	25
Ethoxylated Emulsifiers	Ethoxylated partial glycerides	--
	Polysorbates	25
Phosphatides, Phosphorylated	Lecithin	Not limited
Partial Glycerides	Fractionned phosphatides	--
	Phosphorylated monoglyceride	--
Miscellaneous	Sodium stearoyl-lactylate	20
	Calcium stearoyl-lactylate	20
	Salts of fatty acids (Na, K)	Not limited
	Sodium dodecyl sulfate	

* CFR Code of Federal Regulations. ** Acceptable Daily Intake (ADI) for man

Lecithin is obtained for commercial purposes from oil seeds (in particular soja) and egg yolk. It is a phosphatid and thus a fat-similar substance. In the lecithin molecule phosphoric acid is connected on the one hand to a primary hydroxy group of glycerin bound and on the other hand with choline figure 2.5. Soybean lecithin contains only to a third lecithin. It contains further 15% inositphosphatids and approximately 25% kephalin with other connections. The emulsifying agent effect of the lecithins is based on the fact that hydrophilically and the fatty acid remainders behave the zwitterion structure formed by the phosphate and cholin hydrophob. Lecithin is used e.g. during the margarine production, in order to decrease of the surface tension a fine distribution of the water phase [Baltes 1995].

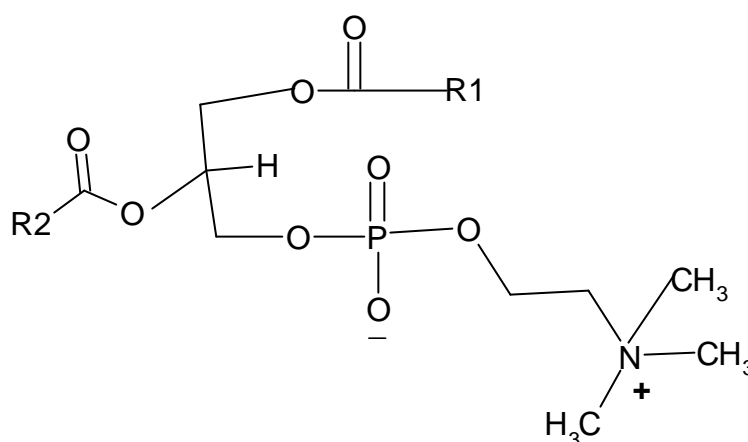


Figure 2.5 : Lecithin (general structure)[Baltes 1995]. R1, R2. typically normal aliphatic remainders with 15 or 17 carbon atoms and up to 4 *cis* -double bounds.

Phosphatidylcholine (PC) is a naturally occurring surfactant which is excellent in terms of biodegradability and human safety. In fact, many PCs have been utilized as fat emulsifiers [Ishii *et al.*, 1990]. However, few studies have been made on preparation and properties of microemulsions with phosphatidylcholine [Kaneko and Shinoda 1988]. None of studies have investigated the potential of non-alcohol cosurfactants to produce microemulsions with lecithin. The most commonly used cosurfactants are simple low molecular weight alcohols [Aboofazeli *et al.*, 1994]. The ability of phospholipids to form microemulsions with alkanes has been studied by several authors [Kaneko and Shinoda 1988, Shinoda *et al.*, 1991]. Leser *et al.*, [1993] have studied the phase behavior and microstructure of soybean phosphatidylcholine, medium chain triglyceride, and short chain alcohols.

Lecithin has a fairly high critical packing parameter (CPP) of around 0.8 [Cornell *et al.*, 1986]. If lecithin containing systems are to form microemulsions over a reasonably wide range of oil and water concentrations, the effective CPP of lecithin needs to be reduced. One way this can be achieved is the addition of a short chain cosurfactant. A short chain cosurfactant can act to reduce the effective CPP in two ways, firstly by its incorporation into the interfacial film and/or secondly by dissolving in the aqueous phase thereby making it less hydrophilic. In addition, in the case of a long hydrophobic chain surfactant like lecithin, a short chain cosurfactant can also act to increase the fluidity of the interfacial surfactant layer, thereby reducing the tendency of lecithin to form highly rigid films [Binks *et al.*, 1989] and allowing the interfacial film sufficient flexibility to take up the different curvatures required to form balanced microemulsions [De Gennes and Taupin 1982].

Soybean lecithin is both strongly hydrophobic, due to long hydrocarbon chains, and strongly lipophobic due to zwitterionic polar head groups. At room temperature soybean lecithin and water form a lamellar liquid crystalline phase between 0.35 and 0.07 mass fraction of water [Bergenstahl and Fontell 1983]. In water-oil systems lecithin is slightly too lipophilic to form microemulsions spontaneously [Backlund *et al.*, 1994]. This means that lecithin need a cosurfactant such as a short-chain alcohol to form microemulsions [Shinoda *et al.*, 1991]

It is important to note that the differences between grades of lecithin are considerably less than the effect of changing the alcohol as cosurfactant [Aboofazli and Lawrence 1994]. The major factor influencing the phase behavior of microemulsions prepared by lecithin as surfactant is the nature and mixing ratio of the cosurfactant used [Aboofazeli *et al.*, 1995].

2.5 Natural colorants

Recently the safety of food colorants has been a very controversial topic but most of the criticism has been directed towards the synthetic colorants. The natural colorants have been relatively free of criticism may be due to the belief that most are derived from food sources that have been consumed for many years [Francis 1996]. According to scientific investigations humans take up the largest part of their information visually. Also, food is assessed first on the basis their outside appearance. A part from form and general presentation the color contributes substantially to the quality assessment by the consumer. For different product groups a different expectation attitude developed concerning the chroma. Generally products with a more intensive coloring are preferred. Processing or storage of a product can lead by oxidative or enzymatic influences to the impairment of the natural colors. In order to arrange the product optically responding, in the context of the legal regulations coloring

materials can be added to food. Other food, which is colorless due to their production process, e.g. lemonade beverages, is colored for the rise of benefit value. The synthetic colorants used in the food-stuffs industry possess an intensive coloring strength with at the same time good solubility, are more stable and easier to process than natural food colorants. The application type of natural food colorants are however so far limited, since they have some disadvantages in comparing with the artificial colorants [Täufel *et al.*, 1993]. They are however relatively safe dealing with toxicological check and incompatibility reactions [Baltes 1995].

Natural colors are organic colorants that are derived from edible sources using reconized food preparation methods. One of the advantages of using natural colors is that they are generally more widely permitted in food-stuffs than synthetic colors. Contrary to many reports, natural sources can provide a comprehensive range of attractive colors for use in the modern food industry. However, although the color of fruits and vegetables can vary during the season and processing can cause color loss, food manufacturers need to ensure uniformity of product appearance [Henry 1996]. Also colors may be added to foods to give color to certain foods such as sugar confectionery, ice lollies and soft trinks, which would otherwise be virtually colorless [Food Advisry Committee, FdAC/REP/4, London 1987].

2.5.1 Chemistry of natural colorants

The coloring of chemical connections is based on the absorption and emission of light. The visible light (approx. 400-800 Nm) is electromagnetic radition, by the wavelength λ and the frequency ν to describe leaves itself (2.1) [Skoog and Leary 1996]:

$$\lambda \cdot \nu = c \quad (2.1)$$

c is the speed of light (in vacuum around $2,99 \cdot 10^{10} \text{ cm} \cdot \text{s}^{-1}$).

$$E = h \cdot \nu \quad (2.2)$$

A light quantum has the energy (2.2) whereby h the Planck`quantum of action is ($6,626 \cdot 10^{-34} \text{ Js}$) [Skoog and Leary 1996]. If light quantum of suitable frequency meets a molecule in the initial state, it can become lively, i.e. suitable electron is raised to a higher energy level.

In order to arrive again into the energy-poorer and thus more stable initial state, the molecule is anxious to deliver the taken up energy again. The put on condition must remain received a certain time, in order to cause coloredness. The material appears in the wavelength the corresponding complementary color. If a connection absorbs e.g. in the violet one (around 420 Nm), then it appears for the human eye yellow [Breitmaier and Jung 1983] figure. 2.6 shows a spectrum of the visible light.

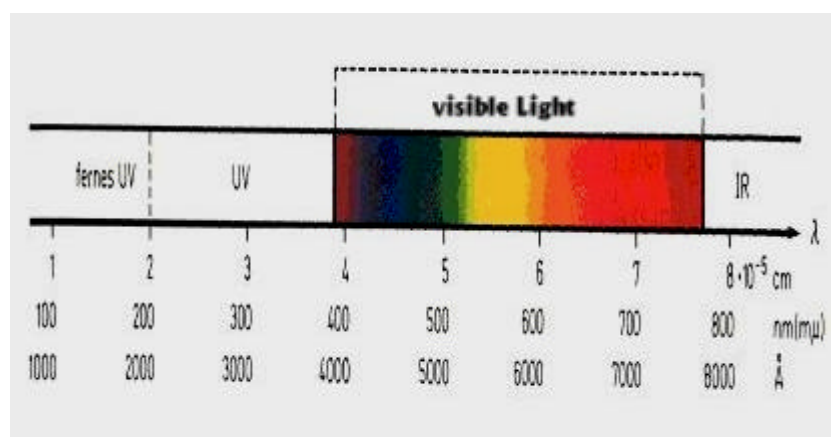


Figure 2.6: Spectrum of the visible light [Breitmaier and Jung 1983].

Regarding the orbit of molecule involved four different electron transitions can be differentiated [Breitmaier and Jung 1983; and Skoog and Leary 1996] (Figure 2.7)

$n \rightarrow \pi^*$ -Transition

If electron of a non-binding a few of electrons becomes lively on an anti-binding π^* -orbital of a double bond, one speaks of a $n \rightarrow \pi^*$ -transition. These transitions are in all connections with hydroxyl groups and neighbouring double bond possible, e.g. carbonyl or azo connections. The $n \rightarrow \pi^*$ -transition probability is only small. Therefore the appropriate absorption bands are likewise small.

$\pi \rightarrow \pi^*$ -Transition

Transition is raised an electron to a π^* -orbital, speaks one of a $\pi \rightarrow \pi^*$ -transition This type from transitions is in all insatiated connections possible, so in alkenes, alkynes, aromatics and further in transition probability is very high. Therefore the appropriate absorption bands are intensive.

$n \rightarrow \pi^*$ -Transition

If n -electrons to anti-binding π^* -orbitals are a neighbouring linkage, one speaks lively of $n \rightarrow \pi^*$ -transitions. Generally, they can be caused by radiation of the region between 150 and 250 Nm. They are in satisfied connections with heteroatomen possible, e.g. with alcohols, amines or with alkyl halides.

$\sigma \rightarrow \pi^*$ -Transition

If σ -electrons become lively to transition in the appropriate anti-binding π^* -orbital, one speaks of $\sigma \rightarrow \pi^*$ -transitions. They are possible in all organic compounds. The energy which can be spent is very large and corresponds to radiation frequencies within the vacuum ultraviolet area.

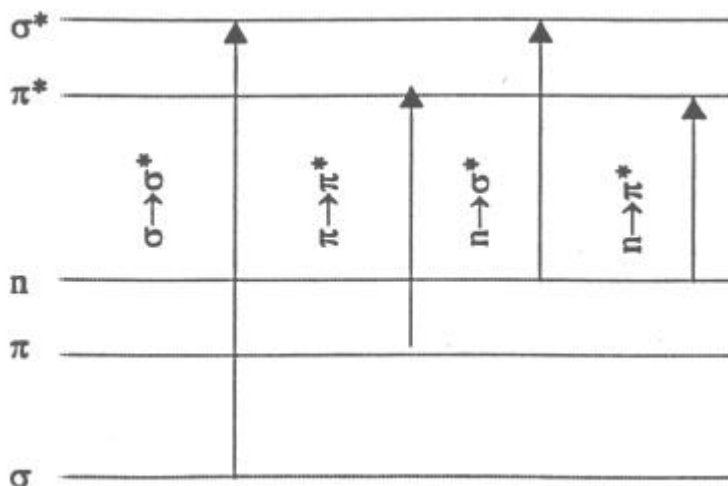


Figure 2.7: Electronic molecule energy levels [Skoog and leary 1996].

Substituents, which shift the absorption to longer-wave light, are called Chromophore (chromophore groups). They possess bonding electrons with relatively low excitation energies. When the absorption capacity is the larger, the electron system is the more expanded. One differentiates two types from chromophors (Figure 2.8).

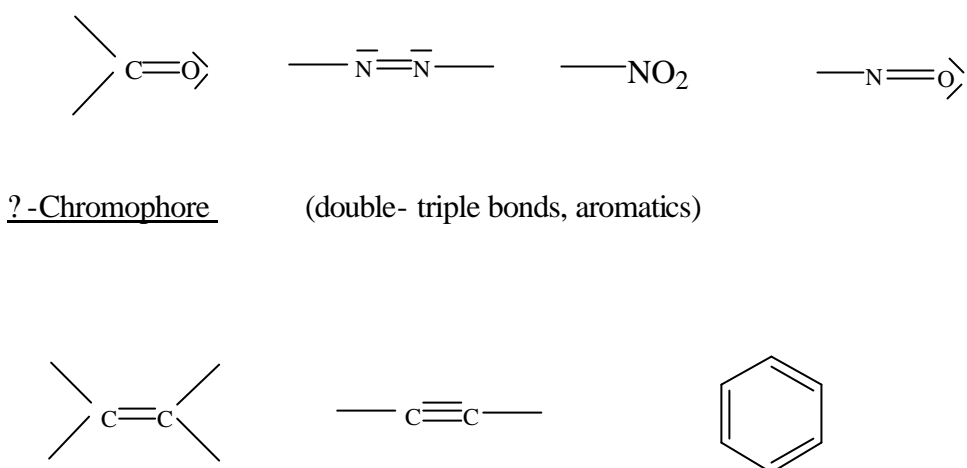


Figure 2.8: Examples for chromophor groups

Usually, it requires several chromophor groups in the molecule, in order to shift the light absorption into the visible spectral region [Täufel *et al.*, 1993]. The conjugation of electrons leads to a further delocalization, which degrades the π^* -energy levels. Consequently the absorption maximal shift to longer wavelengths [Skoog and Leary 1996]. The increase of λ_{max} one defines as red or bathochrom shift. Fewer significantly is the shift absorption maximum to shorter wavelengths. The so-called blue shift is probably due to an increase of solvating the free pair of electrons, whereby the energy of the n-orbital is lowered [Skoog and Leary 1996]. Auxochrome (auxochrome groups) are substituents with n-electrons, however without linkages, which extend the conjugation system of the chromophors and thus for an increase of the chroma contribute. Examples for this are hydroxyl and the amino group (also substitutes) [Schröter *et al.*, 1995].

2.5.2 Stability of natural colorants

Natural colorants are found in many plants and animals. They fulfil different functions e.g. attracting from insects to dusting the blooms. Natural colorings were used since ever ago for coloring (e.g. in the classic antiquity magenta for very valuable textiles). Due to their molecular structure natural colorings are sensitive for the light and oxygen. The conjugation of chromophores (Figure 2.9), frequently occurring in natural colorants, lead with appropriate suggestion by light to the delocalization of π -electrons. From it results a theoretical „turning“ of the double bonds, due to whose different mesomer phases can be trained.

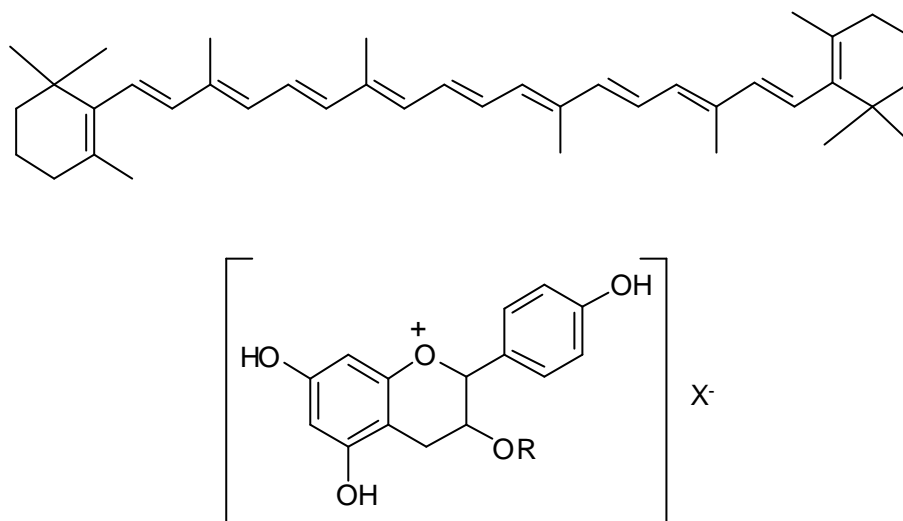


Figure 2.9: Natural colorings (β -Carotin, a Carotinoid; Pelargonidin, an Anthocyanidin if R = H, an Anthocyan if R = sugar remainder, X = halide, usually Cl)

The developing partial charges favors a depositing of oxygen radicals. Thereby the delocalization of the electrons is limited. The consequence is a reduced light absorption readiness and thus a weaker color impression. The reaction mechanism wide on the basis the molecular structure represented by curcumin (Figure 2.10).

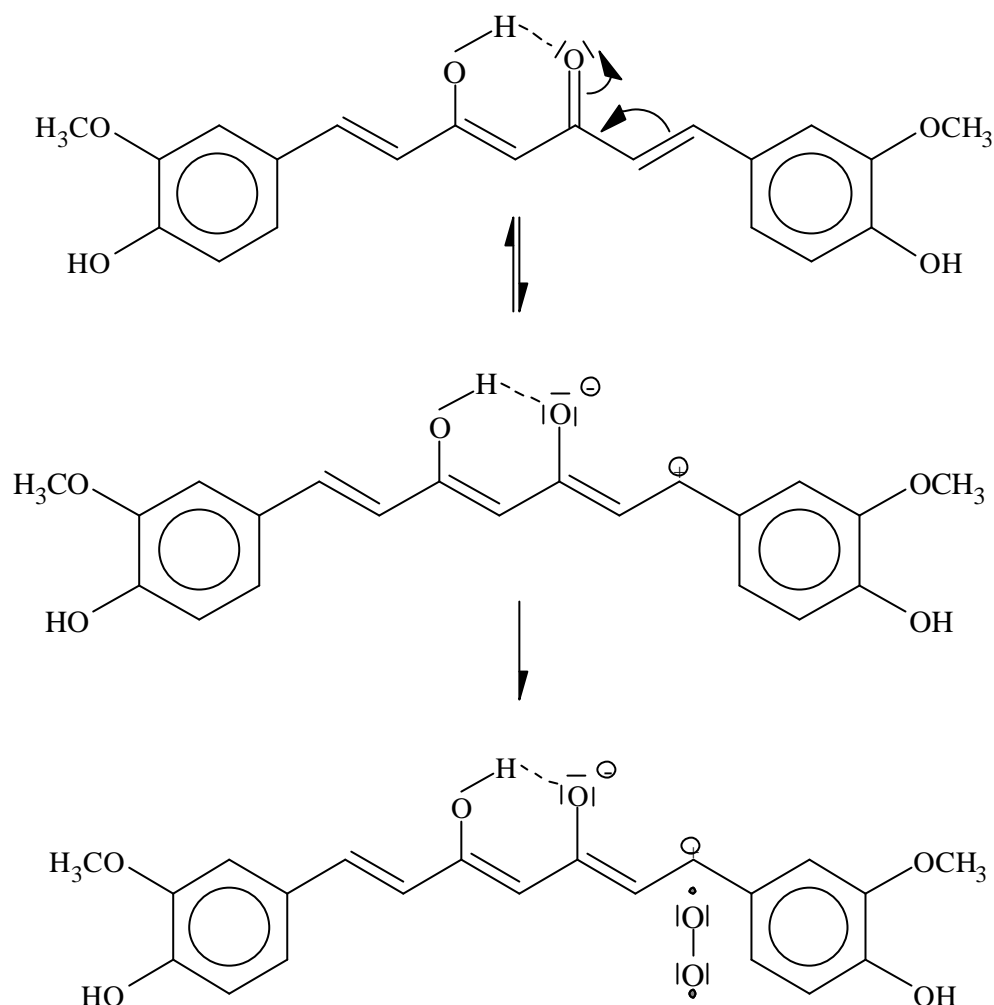


Figure 2.10: Suggestion of natural colorings by UV-light and attack by acidity radical for the example of curcumin.

2.5.3 Curcumin

The yellow coloring curcumin originates from the roots of the turmeric plants resident in South-east Asia (bot.: *Curcuma longa*). It is produced by extraction of the degreased root. Chemical formula of curcumin is $C_{21}H_{20}O_6$, molecular weight is 368.4 and color shade is lemon yellow at pH3, orange at pH10 [Food Advisory Committee, FdAC/REO/4, HMSO, London 1987]. It is discussed that curcumin has an anti-carcinogenic effect [Falbe and Regitz 1990]. Curcumin has been shown to inhibit lipoxygenase and cyclooxygenase [Huang et al., 1991] and thereby inhibits the superoxide generation and prevents tumour promotion [Brian and Cizela 1989]. Curcumin has been successfully used in several experimental carcinogenesis models. Recently, curcumin has also been shown to inhibit protein kinase C, and thereby inhibits oncogene activation [Reddy and Aggarwal 1994].

It has been suggested that the hydroxy groups on the benzene rings, double bonds in the alkene portion of the molecule and/or the central β -diketone moiety could be responsible for the high biological activity of curcumin [Ruby *et al.*, 1995].

Curcumin also increased the level of glutathione which would prevent thiol depletion occurring during apoptosis [Jaruga *et al.*, 1998]. Curcumin is an interesting molecule because of the variety of biological effects. It possesses in addition to its potent anticancer activity. However, its exact mechanism of action is not very clear. It suggested that its anticancer activity could be mainly due to its ability to induce apoptosis in tumor cells [Khar *et al.*, 1999].

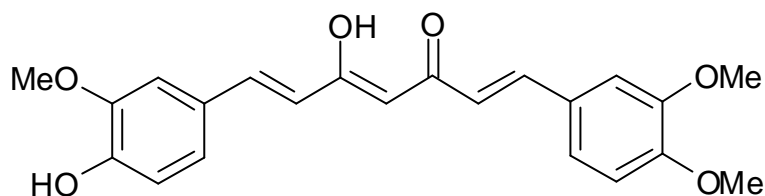


Figure 2.11. Curcumin structure [Baltes 1995].

It has been shown that curcumin has a poor light stability. The stability of curcumin was strongly improved by lowering the pH or by adding glutathione, N-acetyl-L-cysteine, ascorbic acid and rat liver microsomes [Cooper *et al.*, 1994]. The oral LD50 for curcumin in mice is higher than 2g [Srimal and Dhawan 1973]. Monkeys fed 500 mg/kg/day for 9 months of turmeric showed no adverse effects [Bhavanishanker *et al.*, 1986].

The solubility is very different as a function of solvents: Curcumin is soluble in vegetable oils, however insoluble in water, in ethanol solve itself 3 g.L^{-1} . The light fastness is small, particularly in solve form in products with high water activity. The coloring agent is heat-proof until $150 \text{ }^{\circ}\text{C}$. It possesses a good acid resistance. Above a pH value of 9 a color changes from yellow to orange. The molecular structure is represented in figure 2.11. The coloring agent is certified only under marking for certain food. Curcumin uses among other things for the coloring of curry, mustard, sweet goods, eis-crem, fruit and vegetable prepared and fine pastry. The admissible in each case are maximum quantites thereby to consider (guideline 94/36/EG) [Falbe and Regitz 1990]

3 Objectives

In recent years, microemulsions have been given considerable attention due to their special important physicochemical properties such as their transparent or translucent appearance, long term stability, high solubilization capacity for lipophilic and hydrophilic substances, and ease of preparation. Because of that microemulsions are very important in various applications of commercial industries.

Huge number of papers have been published in microemulsion area. Unfortunately, most of work to date studying microemulsions used oils, surfactants and cosurfactants unsuitable for food applications and there are very few available examples of microemulsion systems for this purpose. In order to make these systems, it is necessary to formulate such systems using nontoxic and safe ingredients. The problem is how to prepare microemulsions with harmless oils, surfactants, and cosurfactants for possible applications in food products.

The present investigation aimed to prepare microemulsions by using inexpensive and suitable components for food applications. The microemulsions can be used as medium for water and oil soluble nutrients because they contain both aqueous phase and oil phase. Curcumin is very important since it can be used as anticarcinogenic, natural food colorant and antioxidant. Curcumin is insoluble in water and sensitive towards. For these reasons this study carried out also to investigate the ability of these microemulsions for solubility of curcumin and to study the stability of curcumin solubilized in them against light during storage, making stress on the following items :

- The preparation of various microemulsion systems.
- The determination of curcumin solubility in these systems.
- Study the stability of curcumin solubilized in these microemulsions against light by determination the effect of UV light and normal electric light on its color shade during storage.

4 Materials and Methods

4.1 Materials

4.1.1 Chemicals used in microemulsion preparation

The chemicals generally used in the preparation of microemulsions are four: surfactant, cosurfactant, oil and water. The microemulsions are formed mainly by using at least three components an oil, a water and a surfactant. Surfactants are necessary for the solubility of water in oil, forming therefore a microemulsion. Some surfactants are more soluble in oil than in water or vice versa, in which case the addition of a cosurfactant can assist the obtation of a balanced microemulsion.

4.1.1.1 Surfactants

It is well known that oils are insoluble in water. Surfactant or mixture of surfactant and cosurfactant are usually located at the surface separating two immiscible liquids for the stability of the mutual dispersion [Bourrel and Schechter, 1988]. A surfactant phase with a bicontinuous structure consists of intertwined water and oil lamellae separated by a surfactant layer with low or zero curvature, hence it has the highest mutual solubility between oil and water [Sonesson *et al.*, 1991]. Surfactants are nonionic, ionic or cationic. All surfactants used in this study were nonionic, because as reported by Costantinides the nonionic surfactants are known to be less affected by pH and ionic strength changes [Costantinides and Yiv, 1995] and some data have observed that nonionic surfactants offer the advantage of a high chemical stability [Hofland *et al.*, 1994]. Microemulsions stabilized by nonionic surfactants may need no cosurfactants [Aboofazeli *et al.*, 1994].

Three types of surfactants were used: lecithin, monoolein and Tween20. These surfactants were selected because they are safe for food uses and with the exception of Tween20, the acceptable daily intake (ADI) is not limited. Moreover, they are inexpensive and available compared to other surfactants.

4.1.1.1.1 Lecithin

Phosphatidylcholine is a naturally occurring emulsifier excellent in terms of biodegradability and human safety [Ishii *et al.*, 1990]. In this study 3-sn-phosphatidylcholine (lecithin) from soybean containing 40% phosphatidylcholine was used as surfactant to prepare the microemulsions. It was purchased from Fluka and was used as received. Aboofazeli and Lawrence [1994] have investigated the influence of purity of lecithin on producing balanced microemulsions and they noted that the differences between grades of lecithin are considerably little. Therefore, it is not unreasonable to use lecithin containing 40% phosphatidylcholine to prepare food microemulsions where it is commercially available and inexpensive.

4.1.1.1.2 Tween 20

Tween 20 (Polyoxyethylen-sorbitan-monolaurat) was obtained from Fluka. It was selected because it can be used in food products with acceptable daily intake (ADI) 10 mg/k. It also has a high ability for solubility of aqueous phase in peppermint oil to prepare transparent microemulsion without a cosurfactant. In addition it is able to dissolve curcumin which is used as natural coloring material, easily available and economical.

4.1.1.1.3 Monoolein

Monoglycerides are nonionic surfactants widely used as emulsifiers in the food and pharmaceutical industries [Holmberg and Österberg 1988]. DL- α -Monoolein (Glycerin-1-monooleate) used in this work, consists of 38-40% diglyceride, 16-18% triglyceride and 4-5% free glycerol in addition to the essential component (mono-olein). It was purchased from Fluka. Its Selection was due to the suitability of its uses in food, its low cost and could be used both as surfactant and as oil.

4.1.1.2 Oils

The oils used in this study were vegetable oils such as peppermint, soybean, peanut and rapeseed oil. As reported by Winkler [1992], oils obtained from natural sources and their derivatives, e.g. triglycerides, are easily degraded by microorganisms and are considered to be harmless to the environment. Such microemulsion systems containing these oils are but infrequently published in the literature [Paul and Moulik 1991]. Triglycerides are preferred

as they have been used in intravenous fat emulsions for more than 20 years and are therefore well characterized from the toxicological aspect [[Davis *et al.*, 1983].

4.1.1.2.1 Peppermint oil

This oil is extracted from *Mentha piperta L.*, plant and it was purchased from Fluka. This is a special oil due to its contents of 30-40% menthol, 15-32% menthone and 3-10% menthyl acetate. It was selected for this work because due to its effectiveness in microemulsion preparation by using lecithin or Tween20 as surfactants without or with cosurfactants. It could be used peppermint oil alone or mixed with a common edible oil as lipophilic phase. In addition, it quickens the solubility of lecithin in edible oils where the lecithin solubility is very difficult.

4.1.1.2.2 Edible oils

Edible oils which were used in this work are soybean, peanut and rapeseed oil which were purchased from Fluka. Soybean oil contains 41% saturated and 59% unsaturated fatty acids; peanut oil 17% saturated and 83% unsaturated fatty acids; and rapeseed oil 7% saturated and 93% unsaturated fatty acids. These oils are a suitable oil phase in microemulsions for food applications. Microemulsions containing these oils are important and interesting where there is a demand for environmentally more acceptable formulations.

4.1.1.3 Cosurfactants

The most commonly used cosurfactants are simple low molecular weight alcohols. To date, however, very little work has investigated the use of other cosurfactants. The presence of the cosurfactant in the interfacial region causes the destruction of the liquid crystalline phase (that prevents the formation of microemulsion) thereby allowing the production of balanced microemulsions [Aboofazeli *et al.*, 1994]. Alkanols as cosurfactants dissolve mainly in the interfacial film and thereby, change its properties. Hitherto, two models have been proposed. The first model is based on the “wedge model” suggested first by Langmuir [1916] who assumed that the interfacial layer possesses a natural curvature. On the basis of this model, Mitchell and Ninham [1981] assumed alkanol molecules to intrude the interfacial layer thereby, changing its curvature. In the second model, proposed by De Gennes and Toupin [1982]. It is suggested that the alkanol molecules adsorb preferentially at the region of strong curvature of the interfacial layer, thereby reducing its rigidity [Kahlweit *et al.*, 1995].

The role of cosurfactant together with the surfactant is to lower the interfacial tension down to a very small even transient negative value at which the interface would expand to make fine dispersed droplets, and subsequently adsorb more surfactant and surfactant/cosurfactant until their bulk condition is depleted enough to make interfacial tension positive again. This process known as “spontaneous emulsification“ forms the microemulsion [Kunieda *et al.*, 1988]. In the production of a balanced lecithin microemulsion, the cosurfactant has an additional role in that it can also act to lower the tendency of lecithin to form a highly rigid film [Binks *et al.*, 1989].

Ethanol

Absolute ethanol was obtained from Merck. It was used in this work as cosurfactant to prepare microemulsions in particular by using lecithin or monoolein as surfactant. In addition, using it assists the solubility of lecithin in edible oils. It is known alcohols, in general, are unsuitable for food uses, but using a few amount (about 5%) of ethanol in food products is acceptable [Osborne *et al.*, 1991].

4.1.1.4 Water

Water was used after double distillation because the hard water may to be difficult in the preparation of microemulsions. Water was used as aqueous phase to form most microemulsion systems.

4.1.2 Other Chemicals

4.1.2.1 Curcumin

Curcumin was used in this study as natural coloring material for the determination of its solubility in microemulsions and stability of curcumin solubilized in these microemulsions against light. Curcumin was supplied by Dr. Marcus GmbH.

4.1.2.2 Sodium chloride, Sucrose and Citric acid

These chemicals were obtained from Merck and were used as electrolyte to determine the effect of them on the preparation of microemulsions and also their effect on the solubility and stability of curcumin dissolved in microemulsions.

All the chemicals were used as received.

4.1.3 Equipments and Instruments

4.1.3.1 Lamps

4.1.3.1.1 Normal electric lamp

Type: elite, q 528, 100 W, 230 V. This lamp was used to give a similar light at all times for the samples stored under light and to know the effect of light on the curcumin dissolved in microemulsions during storage.

4.1.3.1.2 Ultraviolet (UV) lamp

Luminous element: OSRAM Dulux L; 24 W / 41. This lamp was used to give Ultraviolet light for the samples stored in order to determine the stability of curcumin solubilized in microemulsions.

4.1.3.2 Color Measuring Instrument

Minolta Chroma-Meter CR-310.

Type: Remissions-color measuring instrument after three scales-processes for object colors.

Measuring receiver: 6 silizium-photodiodes (two flash processes) filtered for the primary color sensitivity values of green, red and blue light.

Light source: Xenon-flash tube (PXA).

Measuring field : 50 mm.

This instrument was used in this study to measure the color shade of curcumin solubilized in microemulsions before and after storage under various light fields.

4.1.3.3 PH-Meter

Type: Knick, pH-Meter 765 Calimatic

It was used to measure pH-value of prepared microemulsions to know the effect of pH-value on the stability of curcumin.

4.1.3.5 Vortex mixer

Type : IKAMAG REO

It was used to shake the mixtures during the preparation of microemulsions. This mixer works by using an electric magnetic Vortex. Another type of mixer (JANKE & KUNKEL, IKA-Labortechnik, VF2) was used to shake the test tube during the solubility of curcumin.

4.2. Methods

4.2.1 Preparation of Microemulsions

Microemulsions were prepared in three systems defined on the basis of surfactant type:

?? Lecithin-microemulsion

?? Monoolein-microemulsion

?? Tween20-microemulsion.

4.2.1.1 Lecithin-microemulsion

Lecithin-microemulsion was prepared by using either peppermint oil or mixture of peppermint and an edible oil as lipophilic phase and water as aqueous phase without or with ethanol as cosurfactant. In case of lecithin-microemulsion containing peppermint oil and water, a three ratio of peppermint oil and lecithin were used (1:1, 2:1 or 3:1). A mass of 10g of lecithin was weighed in a 250 ml beaker and the appropriate amount of peppermint oil (10, 20 or 30g) was added to it. In case of using ethanol, various percentages (2, 4, 6, 8 or 10%) were separately added to lecithin and oil mixture. The beaker was then closed and allowed to stand overnight to dissolve the lecithin in oil. To reduce or shorten the time of lecithin dissolution in oil, the Vortex mixer can be used to shake the mixture. The use of ethanol also assists in mixture time reduction. After dissolving lecithin in oil, the mixture was titrated with water by using a 10 ml syringe equipped with an injection needle (to control the amount of added water) until onset of turbidity. The mixture was shaken after each addition of water for a short time (about 1 min) by hand or by using a Vortex mixer. The amount of water which is used to prepare the microemulsion was determined by weighing before and after the titration. The experiment was carried out at room temperature (20 ± 2 °C). Since only the single phase microemulsion region was of important no attempt was made to obtain the other phases.

Lecithin-microemulsion containing peppermint oil mixed with an edible oil was prepared at three ratios of peppermint oil, edible oil (soybean, peanut or rapeseed oil) and lecithin (1:1:2, 1:1:1 or 1:2:1). In this case, the appropriate amount of lecithin (10g) was weighed in a 250 ml beaker and a fixed amount of peppermint oil (5g in case of ratio 1:1:2 or 10g in case of ratio 1:1:1 and 1:2:1) was added. An amount of edible oil (5g in case of ratio 1:1:2 or 10g in case of ratio 1:1:1 and 1:2:1) was then added to the mixture of peppermint oil and lecithin. In case of using ethanol an appropriate percentage (2, 4, 6, 8, 10, 12 or 14% by weight) was added in this stage. Since lecithin does not easily dissolve in edible oils, and the preparation of microemulsions by using these compounds with lecithin was difficult, the addition of peppermint oil with the proper ratio was important. Moreover, using peppermint oil favours the dissolution of lecithin, likewise is the use of ethanol. In addition, using either peppermint oil or ethanol increase the fluidity of the mixture and reduces the production of foam during the addition of water to lecithin. After incorporating peppermint oil, edible oil and lecithin in the proper ratio (1:1:2, 1:1:1 or 1:2:1) without or with ethanol, the beaker was closed and allowed overnight or shaken by using a Vortex mixer for about 2 hours to dissolve the lecithin in oil. The mixture was then titrated with water by using a 10 ml syringe equipped with an injection needle until the onset of turbidity. The mixture was shaken for a short time (about 2 min) by the hand or by using a Vortex mixer after each addition of water. The amount of water required to prepare a transparent microemulsion was determined by weighing before and after titration.

4.2.1.2 Monoolein-microemulsions

Monoolein-microemulsion systems could not be produced by using monoolein alone or mixed with soybean oil, but the addition of ethanol as cosurfactant is necessary to solubilize the water for the preparation of a transparent and stable solution. Using Tween20 also as another surfactant was applicable for water solubility in monoolein alone without soybean oil. In case of using monoolein alone as surfactant and as oil, an amount of 10g was weighed in a 250 ml beaker and different percentages of ethanol (4, 8, 12, 16% by weight) were singly added to the monoolein. The mixture was then titrated with water by using a syringe equipped with an injection needle and the titration was continued as long as the mixture was transparent. The mixture was shaken for a sufficiently long time after each titration by a Vortex mixer to solubilize the water. The amount of water was determined by weighing the beaker before and after titration. In case of using soybean oil, a fixed amount (5g) was added

to a fixed amount of monoolein (5g) and the different percentages of ethanol (4, 8, 12 or 16%) were then added. The mixture of monoolein, soybean oil and ethanol was titrated with water by using a syringe until turbidity appearance.

The mixture was shaken after each titration of water by hand or by using a Vortex mixer for a sufficient time (about 1 min.). When using Tween20 with monoolein, a desired amount of monoolein (5g) was taken in a 250 ml beaker and a same amount of Tween20 was added. The mixture was titrated with water by using a syringe until the appearance of turbidity. The mixture was shaken after each addition of water for a short time and the maximum amount of water required to form a transparent and stable mixture was determined by weighing the mixture before and after the titration with water. The use of ethanol with the mixture of monoolein and Tween20 lead to the increase of water solubility, in this case, different percentages of ethanol (4, 8, 12 or 16%) were added to the mixture containing 5g of monoolein and 5g of Tween20 and the titration was done with water.

4.2.1.4 Tween20-microemulsions

Tween20-microemulsions were prepared by using peppermint oil as lipophilic phase and water or an aqueous solution of 20% of either NaCl, sucrose or citric acid. The ratios of peppermint oil and Tween20 used in the preparation of microemulsions were 1:1, 1:2, 1:3, 1:4, or 1:5. The aqueous solution of NaCl, sucrose or citric acid was prepared by dissolving 20g of the solid substance in 80g water.

A known weight of peppermint oil (10g) was weighed in a 250 ml beaker and different amounts of Tween20 (10, 20, 30, 40, or 50g) were separately added to make the proper ratio of peppermint oil and Tween20. The mixture of peppermint oil and Tween 20 was then titrated with water or with an aqueous solution which was prepared previously and the titration was continued until production of transparent microemulsion with a maximum amount of water or aqueous solution and before the appearance of turbidity. It is to be emphasized that there is a first turbidity followed by a phase of clearness and then a next turbidity. The mixture was shaken after each addition of aqueous phase for a short time by using a Vortex mixer. The amount of aqueous solution required to prepare microemulsion was calculated by the difference between the weight of the mixture before and after titration.

4.2.2 Solubility of Curcumin

Solubility of curcumin in this research means the maximum amount of curcumin dissolved in the dissolution medium without sediment.

4.2.2.1 Solubility in solvents

Curcumin (1 mg) was added to a test tube with 10g of solvent (water, ethanol, Tween20, peppermint oil, soybean oil,....etc.). Curcumin weight was gradually increased under vigorous shaking effect of a Vortex shaker after each addition for a long time to dissolve the curcumin. The addition of curcumin was continued until the appearance of undissolved curcumin in the bottom of the test tube. The amount of dissolved curcumin was quantified by subtracting the weight of dissolution medium before the addition of curcumin from its weight after dissolving the curcumin.

4.2.2.2 Solubility in mixtures before aqueous phase addition

In this case, a stock solution of oil, peppermint oil alone or mixed with soybean, peanut, or rapeseed oil and a surfactant such as lecithin or Tween20 at the appropriate weight ratio was prepared. This stock solution was used either alone or mixed with a known weight of ethanol. Also, a stock solution of aqueous phase, either water or an aqueous solution of 20% NaCl, sucrose or citric acid and Tween20 was prepared. In all cases, a certain amount of the prepared stock solution (10g) was weighed into a test tube and then a little amount of curcumin (1 mg) was added as described previously.

4.2.2.3 Solubility in microemulsions

First the microemulsion systems were prepared in a 250 ml beaker by using a fixed ratio of oil, surfactant, cosurfactant (if used) and various contents of aqueous phase. A certain weight of microemulsion system (10g) was taken in a test tube and a little weight of curcumin (1mg) was then added and the dissolution was carried out as previous. The dissolved amount of curcumin was estimated by difference between the weight of microemulsion before and after the dissolving of curcumin.

Note : All procedures for preparation of microemulsions and solubilization of curcumin were carried out at room temperature ($20^{\circ}\text{C} \pm 2^{\circ}\text{C}$) also were performed with three repetitions and the average was calculated.

4.2.3 Determination of the pH-value

After the dissolution of curcumin in the microemulsion, 5 g was taken from each sample in a test tube to measure the pH-value by using pH-Meter.

4.2.4 Light stability of curcumin

Stability of curcumin solubilized in microemulsion against light was determined in this work according to the change of color shade (h°) during storage under UV lamp, normal electric lamp or in the darkness.

The description of color by the use of the wavelength alone is unreliable because this method gives insufficient informations. Part of the spectrum cannot be completely absorbed and the rest is scattered or reflexed [Breitmaier and Jung 1983]. Several systems were developed for the precise determination of colors.

The L^* a^* b^* system describes the color by using of three dimensional coordinate systems. The color shade (h°) can be determined from a^* b^* -values according to the following equation:

$$h^\circ = \arctan (b^* / a^*) \quad (4.1)$$

The L^* -value indicates the lightness and it forms a third vertical axis to a^* and b^* axis.

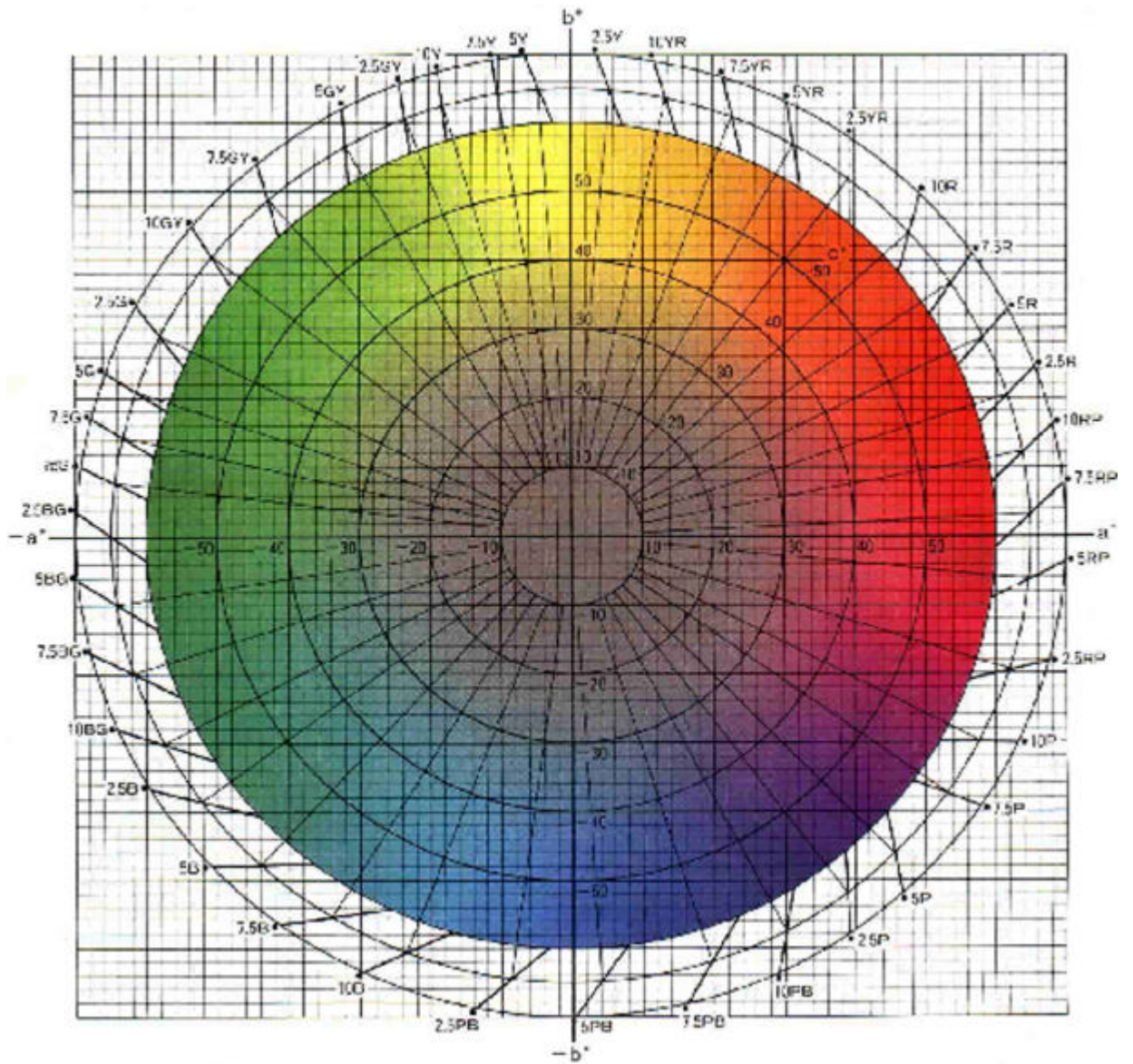


Figure (4.1) shows the a^* and b^* of color slab for the $L^* a^* b^*$ -system.

4.2.4.1 Determination of the color shade

The color shade of curcumin was measured by using the Color Measuring Instrument (L^* a^* b^* -system) according to the above mentioned equation (4.1). The preparation of microemulsion systems was carried out as previous method which was described in section preparation of microemulsions, with exception, the aqueous phase content here was fixed. Such content was 3% in case of lecithin- and monoolein-microemulsion, but in case of Tween20-microemulsions, the aqueous phase content was 11% or 54.5% in ratio 1:1 or 1:4 of peppermint oil and Tween20 respectively. When using the ethanol with the microemulsions based lecithin, the content was 5%, and with the monoolein-microemulsion containing monoolein either alone, mixed with soybean oil or mixed with Tween20, the ethanol content was 10%.

The solubilization of curcumin was performed as described method in section solubility of curcumin in microemulsions with exception the curcumin content here was fixed (0.2% in case of monoolein-microemulsions and 0.4% in other microemulsions

After that, each sample was divided into three parts in a small petri dishes each one containing about 6 g and then the color shade (h°) was measured by using Color Measuring Instrument before storage of the samples (Zero time).

The first part of petri dishes was stored in the dark place and was tightly covered with aluminum seals for 60 days.

The second part was placed under normal electric lamp (100 W) for 60 days

The third was kept under UV lamp (OSRAM Dulux L; 24 W/41) for 30 days.

The places for the second and third parts were equipped with ventilator to remove the warm air by the lamps to avoid generated temperatures increase during storage.

The samples were withdrawn to measure the color shade of curcumin solubilized in microemulsions at gradually increased intervals. The measurements of curcumin color shade were carried out by using Color Measuring Instrument which gives three values L^* , a^* and b^* and from a^* and b^* values the color shade can be calculated according to the above equation (4.1). The petri dishes were covered with their covers during storage and during measuring of hue moreover, they were also covered with additional black cover during the measurement processing.

The resultant value indicates the color of sample such as the range from 105 to 95 means slight yellow, from 95 to 80 is yellow, from 80 to 70 indicate orange, from 70 to 60 is slight red, and from 60 to 45 is deep red as shows in color slab (Figure 4.1). The values of color shade for the samples in this study ranged from 105 to 45.

Concerning the control was selected two mediums for this reason , the first was prepared by using the ethanol diluted with water at 4:1 then the curcumin was solubilized in this medium either at ratio 0.4% and it was used with lecithin-microemulsions or at ratio 0.2% and it was used with monoolein-microemulsions. The second medium of control was prepared by using Tween 20 diluted with water at ratio 1:1 and the curcumin was solubilized at ratio 0.4%and it was used with Tween20-microemulsions.

5 Results

Microemulsions were prepared in three systems defined on basis the type of the surfactant. The first system is lecithin microemulsions which prepared as the following content formulations: lecithin/peppermint oil/water or lecithin/peppermint oil/an edible oil/water without or with ethanol in either cases.

The second is monoolein microemulsions which prepared as the following content formulations: monoolein/water/ethanol, monoolein/soybean oil/water/ethanol or monoolein/Tween20/water without or with ethanol.

The third is Tween20 microemulsions which prepared by the following content formulations: Tween20/peppermint oil/water or Tween20/peppermint oil/an aqueous solution 20% of NaCl, sucrose or citric acid.

Solubility of curcumin was determined in solvents which used in the preparation of microemulsions and also in the prepared microemulsions. Stability of curcumin dissolved in microemulsion systems against light was determined as the change in its color shade (h°) during storage under UV-light, normal electric light or in darkness at room temperature for 30 day in case of UV-light or 60 days in other cases.

5.1 Preparation of microemulsions

5.1.1 Lecithin/peppermint oil microemulsions

Figure (5.1) shows that microemulsion can be prepared by using peppermint oil, lecithin (with various ratio; 1:1, 2:1 and 3:1) and water without or with ethanol. In case of the system free ethanol, maximum amount of water required to prepare microemulsion was 9, 15 and 11 in three various ratios of peppermint oil/lecithin respectively. In case of the system prepared with ethanol, maximum amount of water increased when using ratio 1:1 of peppermint oil and lecithin where was 18% by using 4% of ethanol after that the maximum water decreased with increasing the ethanol. On the other hand, the maximum water decreased in case of ratios 2:1 and 3:1 of peppermint oil and lecithin with increasing the ethanol. The highest amount of water was obtained by using ratio 1:1 of peppermint oil : lecithin (4% of ethanol), while the lowest amount of water was obtained by using ratio 3:1 of peppermint oil : lecithin

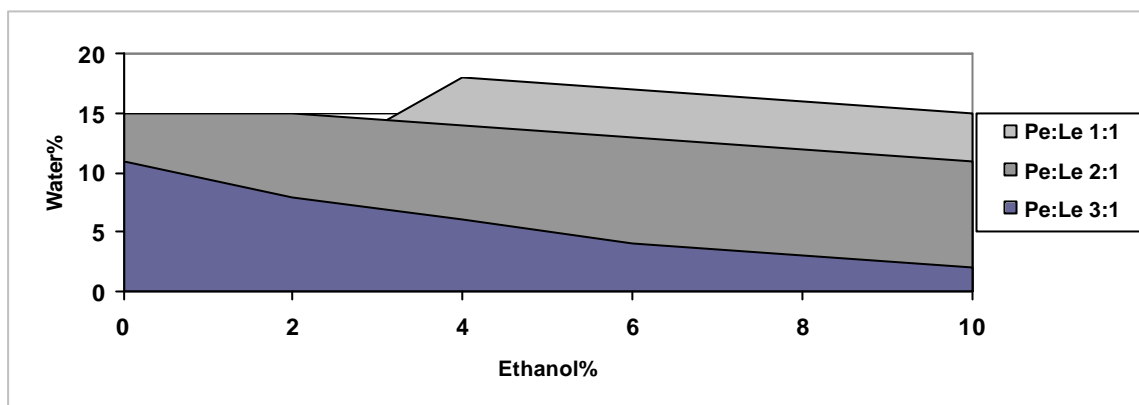
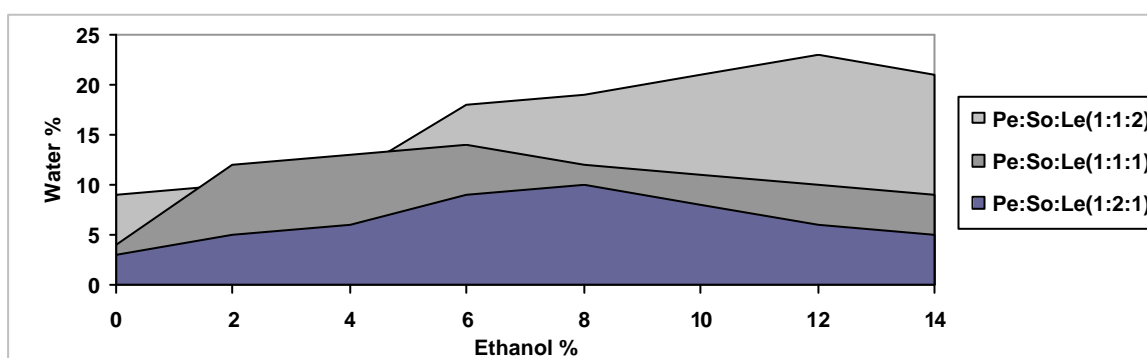


Figure 5.1 Optimum amount of water to prepare microemulsion by using peppermint oil (Pe), lecithin (Le) without or with ethanol

5.1.2 Lecithin/peppermint/soybean oil microemulsions

It should be noted from figure 5.2 that microemulsion was prepared by using water, peppermint oil mixed with soybean oil and lecithin with or without ethanol as cosurfactant. The water content was relatively small in the system free ethanol. On the other hand, when using ethanol the water content increased at first with increasing the ethanol content in three ratios of peppermint oil/soybean oil/lecithin, after that decreased, where it reached 23%, in case of ratio 1:1:2 and using 12% of ethanol, and 17% in case of ratio 1:1:1 and using 6% of ethanol and 12% in ratio 1:2:1 and using 8% of ethanol after that decreased. It should be also shown that the highest water content was by using ratio 1:1:2 and the lowest content was by using ratio 1:2:1 of peppermint oil/soybean oil/lecithin either with or without



ethanol.

Figure 5.2 Optimum amount of water to prepare microemulsion by using peppermint oil (Pe), soybean oil (So) and lecithin (Le) without or with ethanol.

5.1.3 Lecithin/peppermint oil/peanut oil microemulsions

From the results given in figure 5.3 shows that the water content required to prepare microemulsion without ethanol was small in case of ratios 1:1:1 and 1:2:1 of peppermint oil/peanut oil/lecithin, and it was somewhat higher in case of ratio 1:1:2. On the other hand, by using ethanol the water content increased at the first with increasing the ethanol where the maximum contents of water were 20%, 14% and 13% in case of ratio 1:1:2, 1:1:1 and 1:2:1, by using 12%, 10% and 6% respectively, after that they decreased with increasing the ethanol.

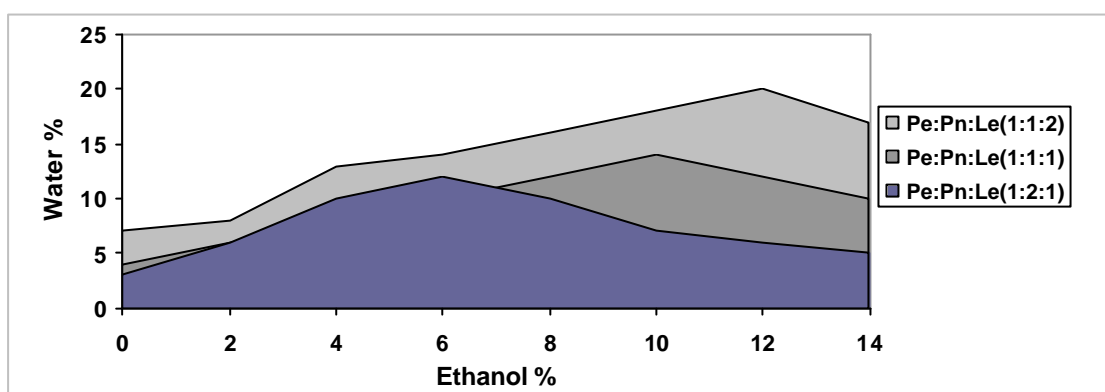


Figure 5.3 optimum amount of water to prepare microemulsion by using peppermint oil (Pe), peanut oil (Pn) and lecithin (Le) without or with ethanol.

5.1.4 Lecithin/peppermint oil/rapeseed oil microemulsions

The microemulsion could be prepared by using peppermint oil combined with rapeseed oil as lipophilic phase, water and lecithin with or without ethanol as cosurfactant. The water contents by using three ratios of peppermint oil, rapeseed oil and lecithin (1:1:2, 1:1:1 and 1:2:1) with or without ethanol were illustrated in figure 5.4. It should be observed that the water content in system free ethanol was smaller in case of ratio 1:2:1 than that in other ratios. By using ethanol, the water content increased at the first, after that decreased with increasing the ethanol in all ratios of peppermint oil/rapeseed oil/lecithin. It should be noted also that the maximum content of water was obtained by using ratio 1:1:2 and by using 12% of ethanol. While the maximum contents in ratios 1:1:1 and 1:2:1 were obtained by using 10% and 14% of ethanol respectively.

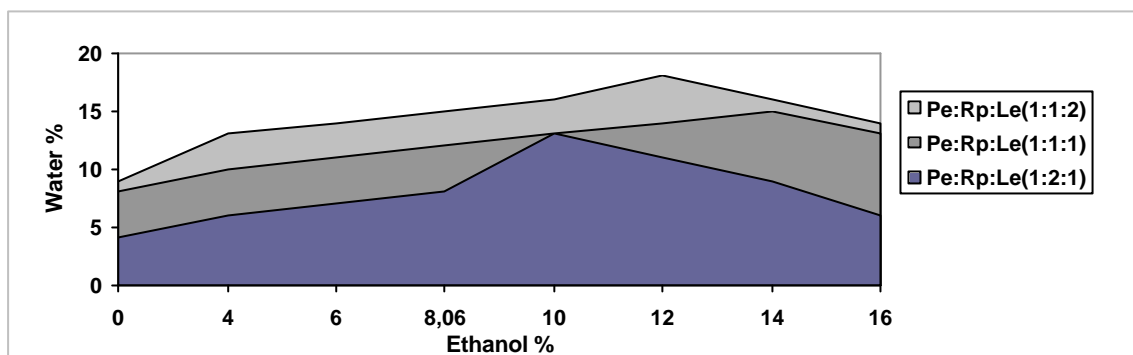


Figure 5.4 Optimum amount of water to prepare microemulsion by using peppermint oil (Pe), rapeseed oil (Rp) and lecithin (Le) without or with ethanol.

5.1.5 Monoolein microemulsions

The data given in figure 5.5 indicated that the transparent microemulsion can be produced by using water, monoolein and ethanol. It can be also produced when mixing the monoolein with soybean oil or Tween20 at ratio 1:1 and ethanol. It should be noted that in case of mixing monoolein with Tween20, the microemulsion can prepared without ethanol but in other cases the addition of ethanol was necessary. The optimum amount of water required to prepare microemulsion was markedly higher in case of using Tween20 than that in case of using monoolein alone or mixed with soybean oil, however in case of using soybean oil it was the lowest one.

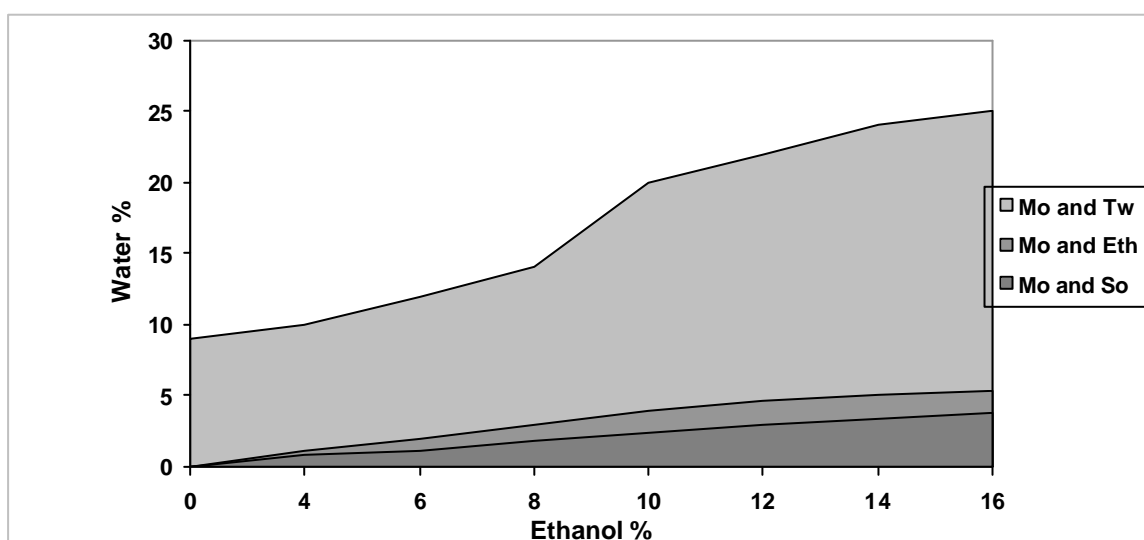


Figure 5.5 Optimum amount of water to prepare microemulsion by using ethanol (Eth) and monoolein (Mo) alone, mixed with soybean oil (So) or Tween20 (Tw) at ratio 1:1.

From this figure shows also that the maximum amount of water increased with increasing the ethanol in all systems but this increment was remarkable in case of using Tween20, likewise at the first the increment was higher than that after addition 12% of ethanol.

5.1.6 Tween20 microemulsios

The microemulsions could be prepared by using peppermint oil, Tween 20 as surfactant and an aqueous solution without cosurfactant as shown in figure 5.6. The aqueous solutions which used were water, sucrose 20%, NaCl 20% and citric acid 20 %. It should be noted also that the microemulsion prepared by using various ratios of peppermint oil and Tween 20. The aqueous solution content increased strongly with increasing of Tween20 and the highest content of aqueous solution was obtained in case of sucrose solution, while the lowest content was obtained when using of NaCl 20% as aqueous solution and in case of water or citric acid the aqueous solution was approximatly similar. The differences of aqueous solution content between all cases of using aqueous solution were small.

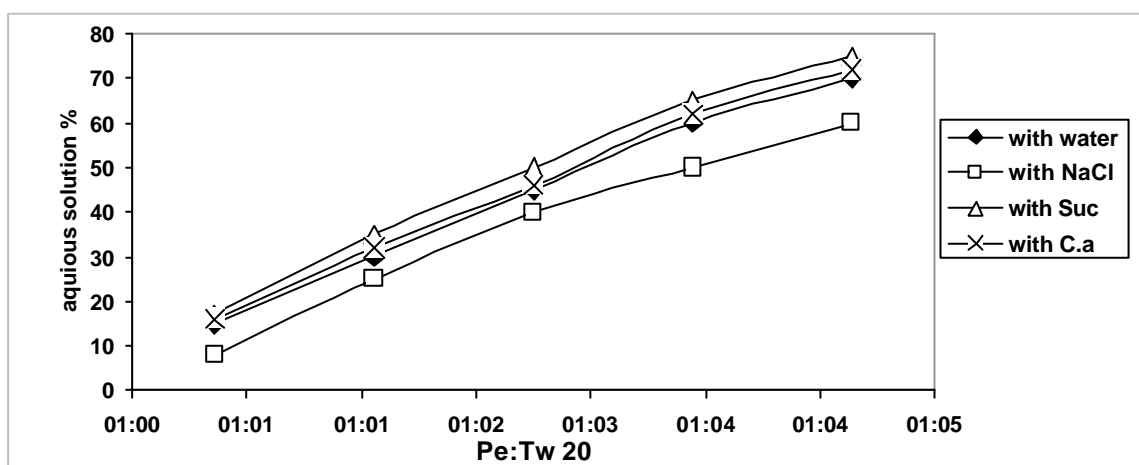


Figure 5.6 Optimum amount of aqueous solution to prepare microemulsion by using peppermint oil (Pe) and Tween20 (Tw) at various ratios. NaCl=sodium chlorid, Suc=sucrose and C.a=citric solution 20%.

5.2 Solubility of Curcumin

5.2.1 Curcumin solubility in solvents

The solubility of curcumin in each solvent used to prepare the microemulsion systems (water, peppermint oil, soybean oil, peanut oil, rapeseed oil, ethanol, monoolein and Tween20) was presented in table 5.1. From data in this table it can be showed that curcumin could not be dissolved in the water, and aqueous solutions of 20% NaCl, sucrose and citric acid. In case of soybean, peanut or rapeseed oil it dissolved slightly where the solubility was 0.02% in these oils. The solubility of curcumin was more in case of peppermint oil, ethanol and monoolein than that in previous components but the maximized solubilization was achieved in case of Tween 20, where it reached 6 %.

Table 5.1 Solubility of Curcumin in solvents used to produce the microemulsions

Component	water	So	Pn	Rp	NaCl	Suc	C.a	Eth	Pe	Mo	Tw20
curcumin %	0.00	0.02	0.02	0.02	0.00	0.00	0.00	0.45	0.55	0.25	6.00

So=soybean oil, Pn=peanut oil, Rp=raps oil, Suc=sucrose solution 20%, C.a=citric acid solution 20%, Eth=ethanol, Pe=peppermint oil, Mo=monoolein and Tw20=Tween 20.

5.2.4 Solubility of curcumin in microemulsions

5.2.2.1 Curcumin solubility in lecithin/peppermint oil microemulsions

From the results in figures 5.7 and 5.8 shows that the solubility of curcumin in lecithin/peppermint oil/water (without or with ethanol) microemulsions ranged between 1.2 and 0.6. The solubility in case of ratio 2:1of peppermint oil and lecithin was somewhat higher than those in other ratios and when using ethanol the solubility was slightly higher than that without ethanol. From the same figures can be noted that the solubility of curcumin decreased gradually with increasing the water content.

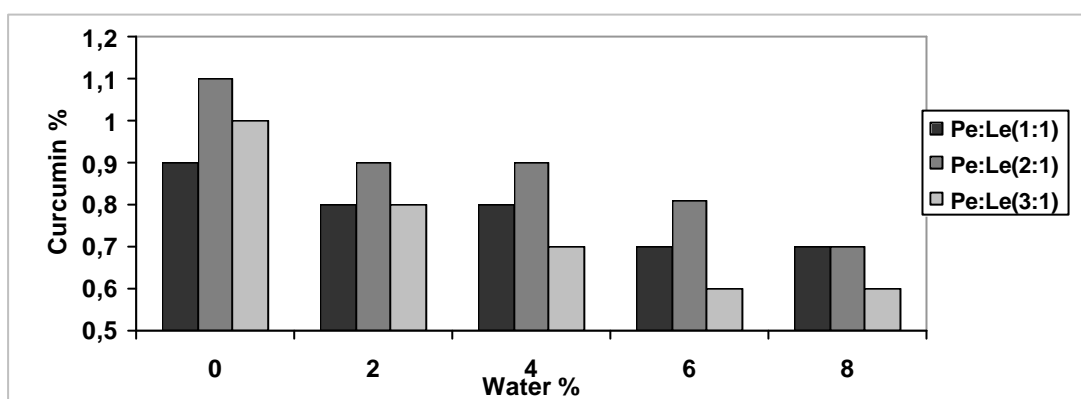


Figure 5.7: Effect of water content on the solubility of curcumin in peppermint oil (Pe)/lecithin (Le) microemulsions without ethanol .

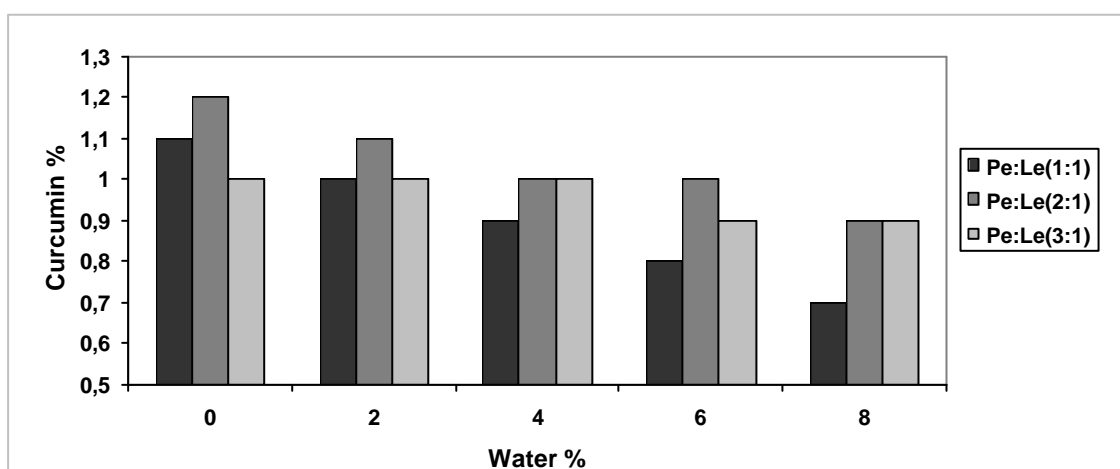


Figure 5.8: Effect of water content on the solubility of curcumin in peppermint oil (Pe)/lecithin (Le) microemulsions with 5% ethanol.

5.2.2.2 Solubility in lecithin/peppermint oil/soybean oil microemulsions

The results in figures 5.9 and 5.10 indicated that the solubility of curcumin in microemulsions containing water, peppermint oil, soybean oil and lecithin without or with ethanol ranged between 0.6 and 0.3%. In case of microemulsions prepared without ethanol the solubility firstly increased and no more increase with increasing the water content until 2% after that decreased in case of ratios 1:1:2 and 1:1:1 of peppermint, soybean and lecithin but in case of ratio 1:2:1 the solubility remained the same with increasing the water content. On the other hand, when using of ethanol the solubility of curcumin increased with addition of 2% water and no change with increasing the water content in case of ratio 1:1:2 of peppermint oil, soybean oil and lecithin, while in case of ratios 1:1:1 and 1:2:1 the solubility was stable with addition of 2% water and then decreased with increasing the water content. From figure 5.9 can be observed that the solubility of curcumin was higher in case of ratio 1:1:2 than those in case of ratios 1:1:1 and 1:2:1.

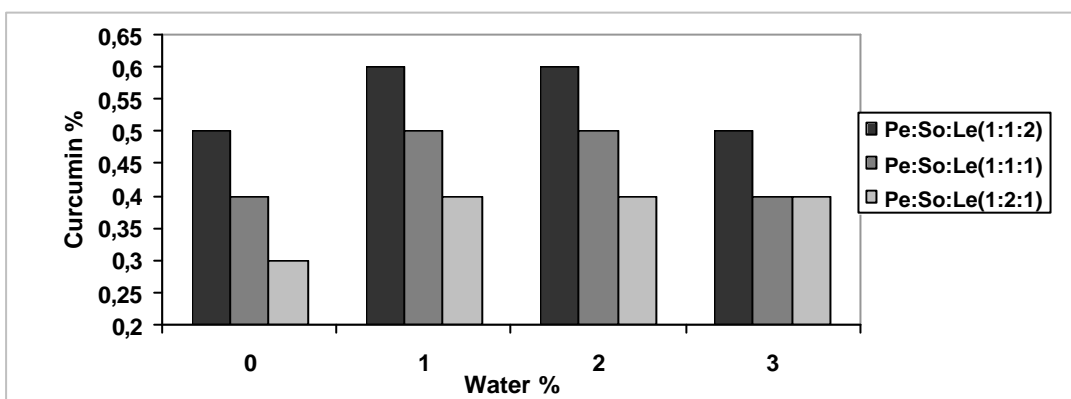


Figure 5.9 Effect of water content on the solubility of curcumin in peppermint oil (Pe)/soybean oil (So)/lecithin (Le) microemulsions without ethanol.

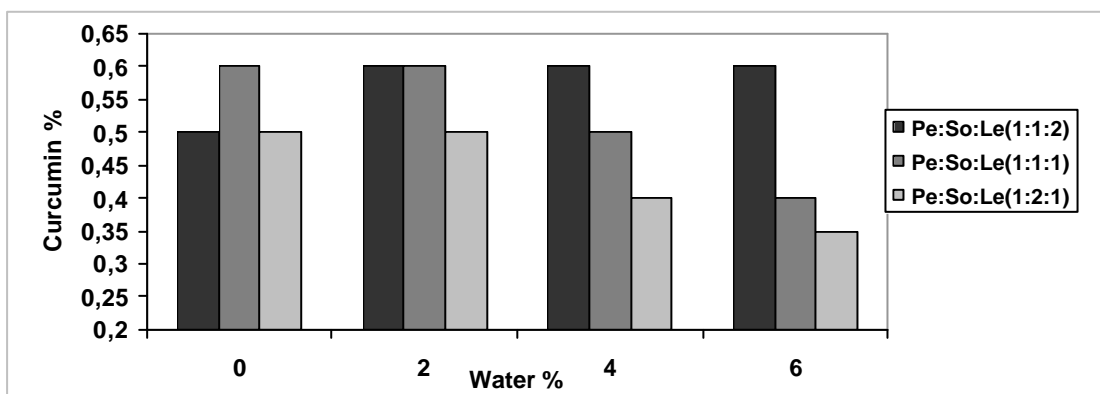


Figure 5.10 Effect of water content on the solubility of curcumin in peppermint oil (Pe)/soybean oil (So)/lecithin microemulsions with 5% ethanol.

5.2.2.3 Solubility in lecithin/peppermint oil/peanut oil microemulsions

The results illustrated in figures 5.11 and 5.12 showed that the solubility of curcumin in microemulsions containing water, peppermint oil, peanut oil and lecithin without or with ethanol ranged between 0.6 and 0.4%. In case of microemulsions prepared without ethanol the solubility increased with addition of 1% water and no more increase with increasing the water content to 2% after that decreased especially in case of ratios 1:1:1 and 1:2:1 of peppermint oil, peanut oil and lecithin, while in case of ratio 1:1:2 the solubility decreased with addition 1% of water and stabilized with increasing the water content.

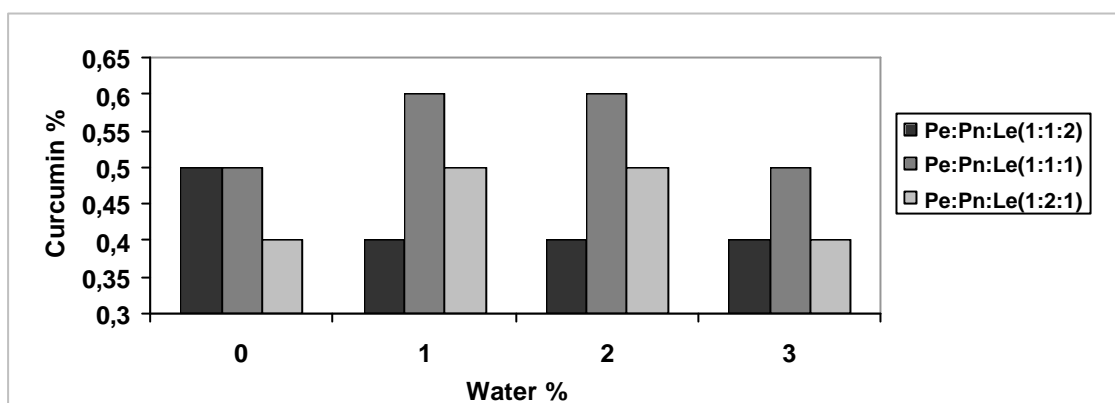


Figure 5.11 Effect of water content on the solubility of curcumin in peppermint oil (Pe)/peanut oil (Pn)/lecithin (Le) microemulsions without ethanol.

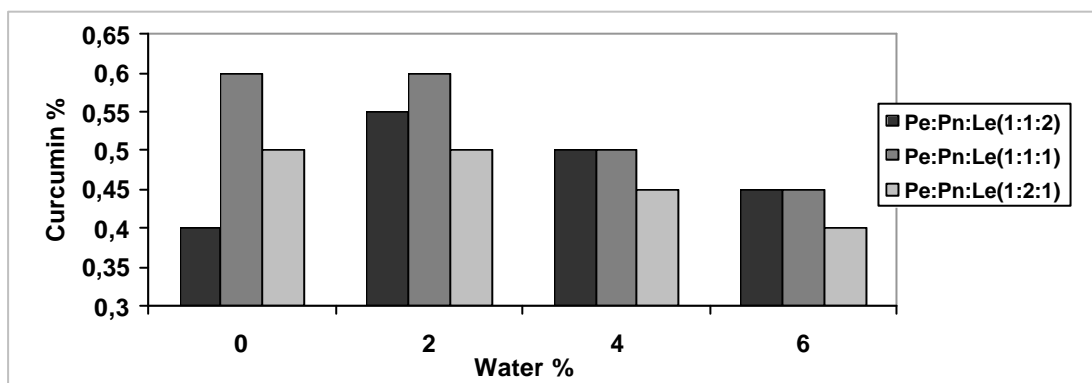


Figure 5.12 Effect of water content on the solubility of curcumin in peppermint oil (Pe)/peanut oil (Pn)/lecithin (Le) with 5% ethanol.

On the other hand, when using ethanol, the solubility of curcumin in case of ratio 1:1:2 increased when addition of 2% water and then decreased with increasing the water. In case of ratios 1:1:1 and 1:2:1 as shows in figure 5.12.

5.2.2.4 Solubility in lecithin/peppermint oil/rapeseed oil microemulsions

As shown in figures 5.13 and 5.14, the solubility of curcumin in these systems ranged between 0.6 and 0.3. From figure 5.13, it is apparent that, in the case of not using ethanol, the solubility in the case of ratios 1:1:1 and 1:2:1 of peppermint oil, rapeseed oil, and lecithin was stable after adding 1% water and then decreased gradually with increasing water. Reversible results were found in the case of ratio 1:1:2 where the solubility somewhat increased with increasing water. On the other hand, when using ethanol and ratios 1:1:1 and 1:2:1 (peppermint oil/rapeseed oil/lecithin), the solubility decreased after adding water until it reached a water content of 4%, after which it stabilized with increasing water to 6% as seen in figure 5.14. In the case of ratio 1:1:2, the solubility decreased after adding 2% water and the reduction continued with increasing water content as shown in the same figure.

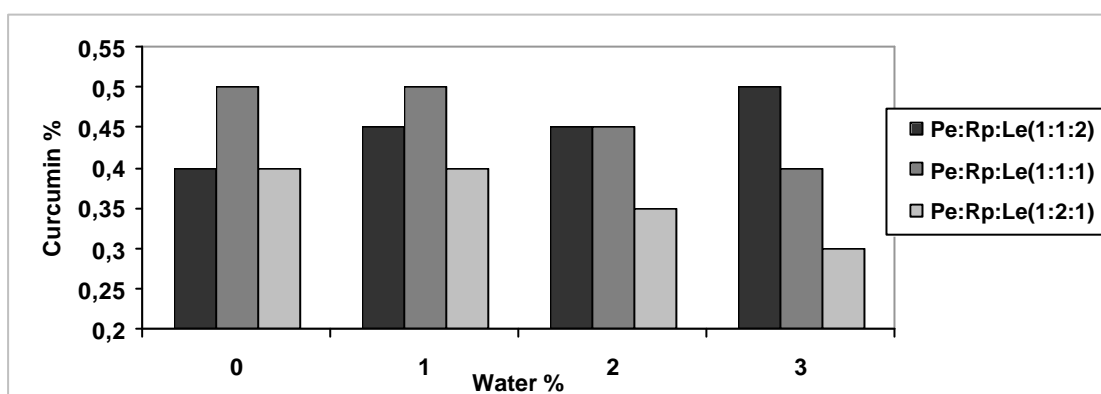


Figure 5.13 Effect of water content on the solubility of curcumin in peppermint oil (Pe)/rapeseed oil (Rp)/lecithin (Le) microemulsions without ethanol.

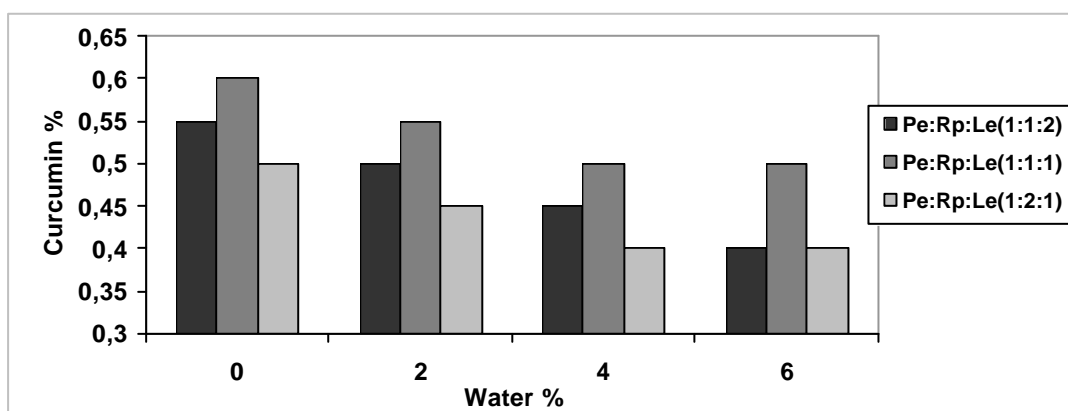


Figure 5.14: Effect of water content on the solubility of curcumin in peppermint oil (Pe)/rapeseed oil (Rp)/lecithin with 5% ethanol.

5.2.2.5 Solubility in monoolein microemulsions

Figure 5.15 showed that the solubility of curcumin in microemulsions containing monoolein and 10% ethanol or monoolein mixed with soybean oil at ratio 1:1 and 10% ethanol was relatively small where it ranged between 0.35 and 0.2, and in case of mixing soybean oil with the monoolein the solubility was lower than that in case of monoolein without soybean oil.

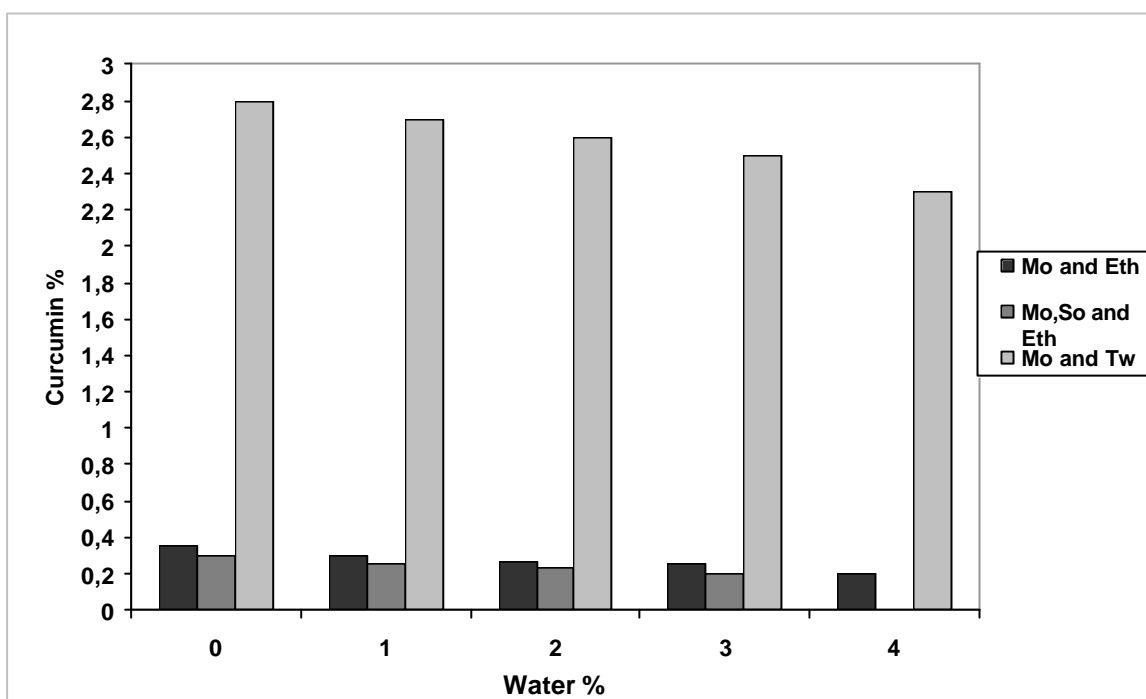


Figure 5.15 Effect of water content on the solubility of curcumin in monoolein microemulsions: monoolein (Mo)/ethanol (Eth), monoolein (Mo)/soybean oil (So)/ethanol (Eth) and monoolein (Mo)/Tween20 (Tw).

On the other hand, in case of system prepared by using monoolein and Tween20 at ratio 1:1 was considerably great in comparing to that in case of monoolein either alone or mixed with soybean oil where ranged between 2.8% and 2.3%. The same figure showed that the solubility decreased gradually with increasing the water content in all systems.

5.2.4.6 Solubility in peppermint oil/Tween20 (1:1) microemulsions

The results presented in figure 5.16 indicated that the solubility of curcumin in microemulsion containing peppermint oil, Tween 20 at ratio 1:1 and water, sodium chlorid 20%, sucrose 20% or citric acid solution 20% was high in comparing with previous systems of microemulsions, where it ranged between 3.5 and 2.5%.

The same figure indicated that the solubility was highest in case of citric acid solution while it was lowest in case of water. Apparent also from this figure that the solubility decreased slightly after addition of 2% aqueous solution and with increasing the aqueous solution it decreased gradually.

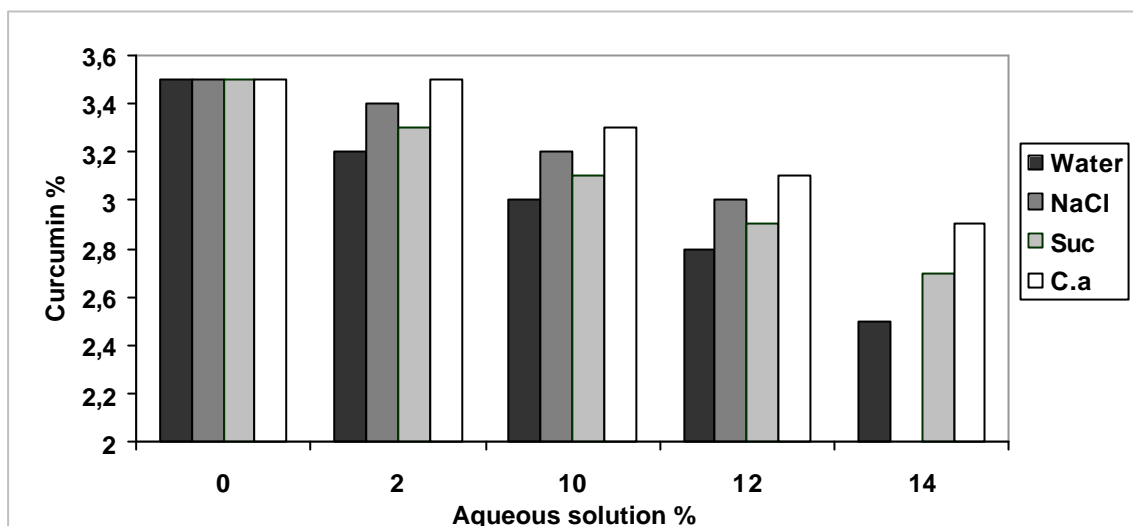


Figure 5.16: Effect of aqueous solution content on the solubility of curcumin in peppermint oil/Tween20 (1:1) microemulsions. NaCl=sodium chlorid, Suc=sucrose and C.a=citric acid solution 20%.

5.2.2.7 Solubility in peppermint oil/Tween20 (1:4) microemulsions

Microemulsions in this system were prepared by using peppermint oil/Tween20 at ratio 1:4 and aqueous phase (water or 20% solution of NaCl, sucrose or citric acid). The results given in figure 5.17 showed that the solubility of curcumin in this system was markedly high in comparing with that in other prepared microemulsions, where the solubility in this case was about 6% before addition the aqueous solution and after addition of 5% aqueous solution decreased to about 4%. It could be noted also that the solubility of curcumin decreased gradually with increasing the aqueous solution. The differences between the solubility of curcumin in all cases of aqueous solution were small.

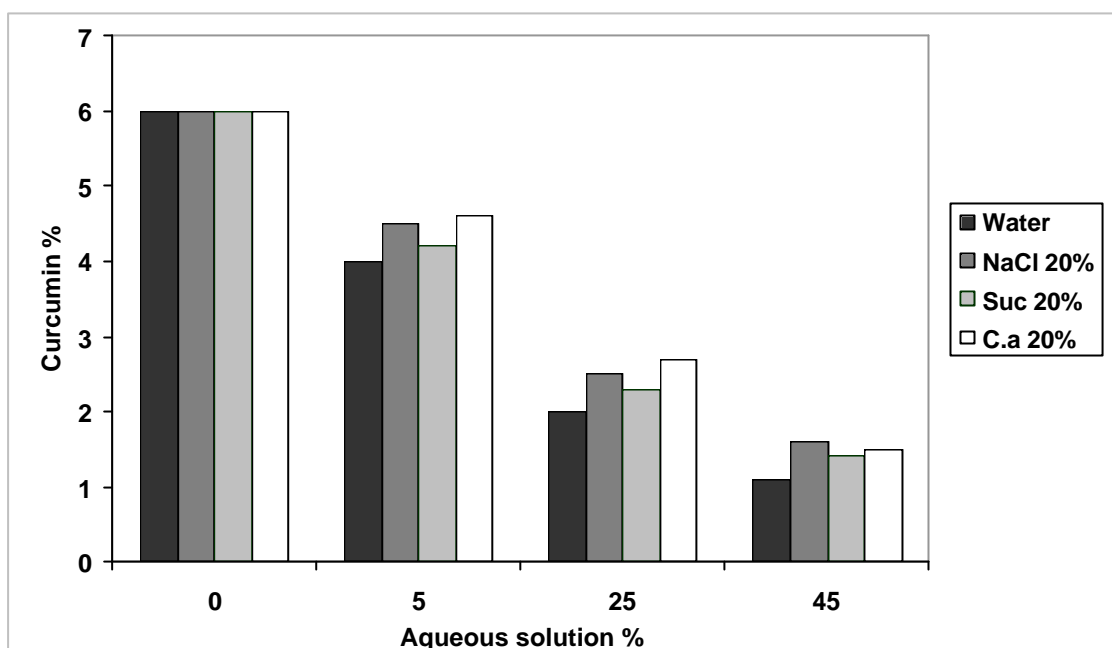


Figure 5.17 Effect of aqueous solution content on the solubility of curcumin in peppermint oil/Tween20 (at ratio 1:4) microemulsions. NaCl=sodium chlorid, Suc=sucrose and C.a=citric acid solution 20%.

5.3 Determination of pH value

The pH-value in microemulsions was measured and the data presented in table 5.2. From this table it should be noted that the pH-value in MEs which prepared by using peppermint oil (or mixed of peppermint oil with an edible oil), lecithin and water ranged between 5.00 and 5.30 while in case of microemulsions containing monoolein (or mixed of monoolein with soybean oil), ethanol and water it was 6.56 (or 6.85). In case of microemulsions containing peppermint oil, Tween20 at ratio 1:1 and water or sucrose solution the pH-value was somewhat high where it was 7.8 or 7.4 respectively but in case of microemulsions containing peppermint oil, Tween20 at ratio 1:4 and water or sucrose solution was 6.80. From the same table apparent that the pH-value in case of using NaCl solution as aqueous phase, peppermint oil and Tween20 either at ratio 1:1 or 1:4 was 6.50 or 6.20 while in case of using the citric acid solution as aqueous phase to prepare microemulsions with peppermint oil and Tween20 either at ratio 1:1 or 1:4 the pH-value was small where it was 3.70 or 2.10.

Table 5.2 the pH-values of microemulsions

System of microemulsions	pH-value	System of microemulsions	pH-value
Pe,Le 1:1 and water	5.30	Pe,Rp,Le 1:2:1 and water	5.00
Pe,Le 2:1 and water	5.26	Mo,Eth and water	6.56
Pe,Le 3:1 and water	5.20	Mo,So 1:1,Eth and water	6.85
Pe,So,Le 1:1:2 and water	5.30	Pe,Tw20 1:1 and water	7.80
Pe,So,Le 1:1:1 and water	5.27	Pe,Tw20 1:1 and NaCl	6.50
Pe,So,Le 1:2:1 and water	5.17	Pe,Tw20 1:1 and Suc	7.40
Pe,Pn,Le 1:1:2 and water	5.30	Pe,Tw20 1:1 and C.a	3.70
Pe,Pn,Le 1:1:1 and water	5.25	Pe,Tw20 1:4 and water	6.80
Pe,Pn,Le 1:2:1 and water	5.00	Pe,Tw20 1:4 and NaCl	6.20
Pe,Rp,Le 1:1:2 and water	5.30	Pe,Tw20 1:4 and Suc	6.80
Pe,Rp,Le 1:1:1 and water	5.20	Pe,Tw20 1:4 and C.a	2.10

Pe=peppermint oil, Le=lecithin, So=soybean oil, Pn=peanut oil, Mo=monoolein, Tw20=Tween20, Eth=ethanol, NaCl=sodium chlorid, Suc=sucrose and C.a=citric acid

5.4 Light stability of curcumin in microemulsions

Stability of curcumin solubilized in microemulsions against light was determined according to the change in color shade (h°) during storage the samples under UV light for 30 days, in the darkness or under normal electric light for 60 days. The color shade was measured by using Measuring Color Instrument.

5.4.1 Light stability in lecithin/peppermint oil microemulsions

Lecithin microemulsions containing peppermint oil as lipophilic phase were prepared at three ratios 1:1, 2:1 or 3:1 Of peppermint oil and lecithin, and 3% water without or with 5% ethanol. An aqueous solution 80% of ethanol was used as control. Curcumin was solubilized in microemulsion samples and in control at concentration 0.4%. The samples and control containing curcumin were stored under UV light, in darkness or under normal electric light and the stability of curcumin was determined as the change in its color shade during the storage.

5.4.1.1 Effect of UV light

Figures 5.18 and 5.19 shows that the changes in color shade of samples either without or with ethanol were very slight but in control the changes were relatively great where the color shade decreased gradually from 90 to 45. This Indicated that the color of curcumin solubilized in the samples was stable (yellow) while it in control changed from yellow to red. Apparent from the same figures that the changes in color of curcumin solubilized in microemulsions containing peppermint oil and lecithin at all ratios were similar.

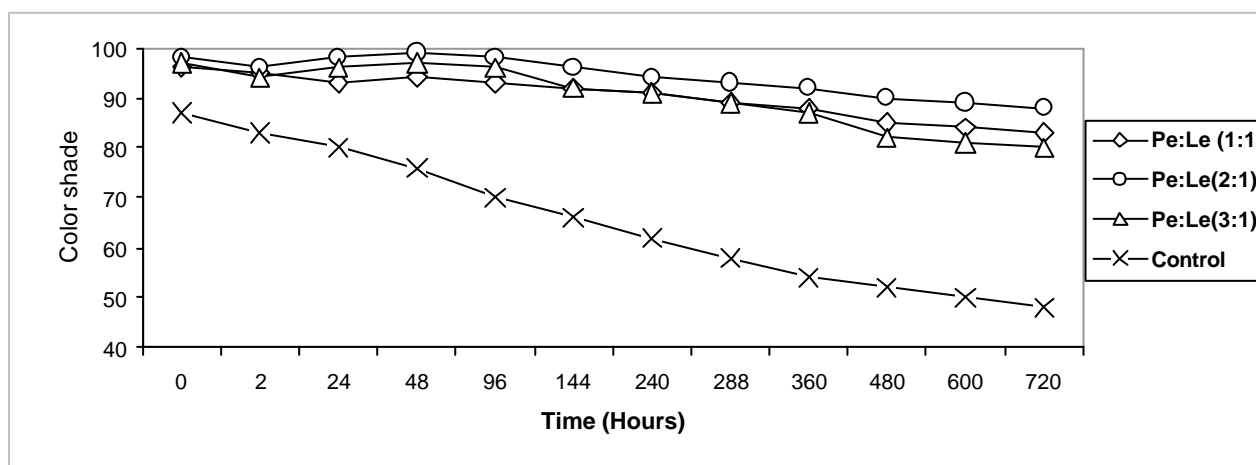


Figure 5.18 Changes in color shade of lecithin (Le)/peppermint oil (Pe) microemulsions containing curcumin during storage under UV light.

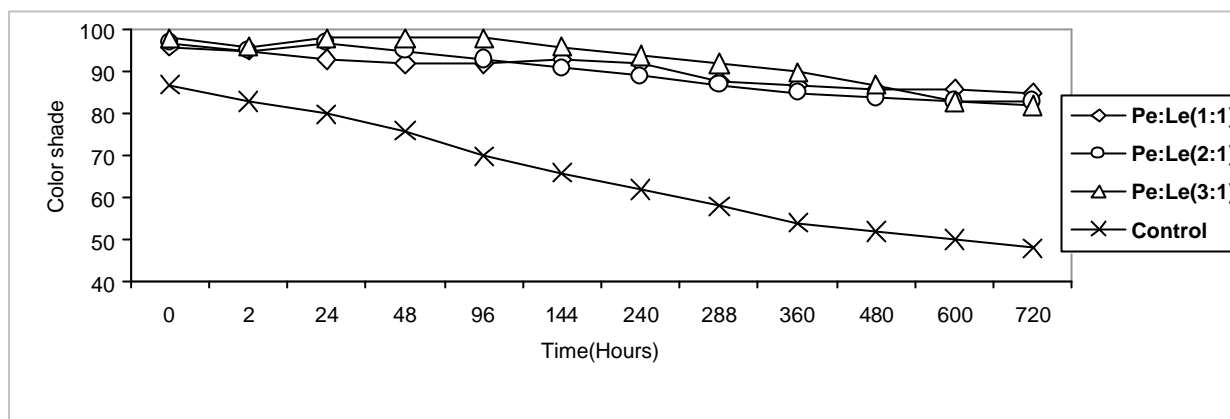


Figure 5.19 Changes in color shade of lecithin (Le)/peppermint oil (Pe)/5% ethanol microemulsions containing curcumin during storage under UV light.

5.4.1.2 Effect of darkness

As showed in figures 5.20 and 5.21 the changes in hue of samples either without or with ethanol were unremarkable, but in case of control, these changes were small where the color shade decreased from 90 to 82. These results shows that the color of curcumin solubilized in control or in microemulsions stored in darkness was somewhat stable. It should be also noted that the changes in color shade were similar in all ratios of peppermint oil and lecithin which used to prepare the microemulsion.

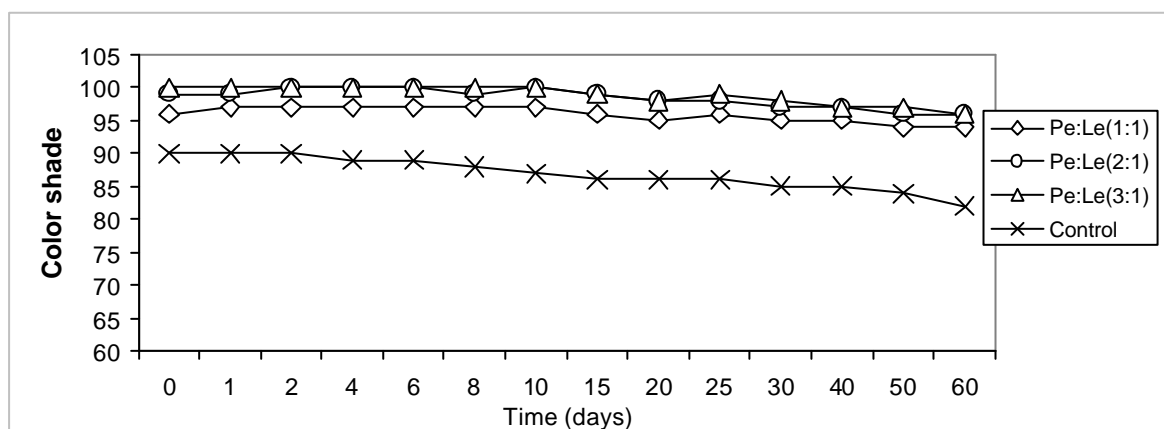


Figure (5.20) Changes in color shade of lecithin (Le)/peppermint oil (Pe) microemulsions containing curcumin during storage in darkness.

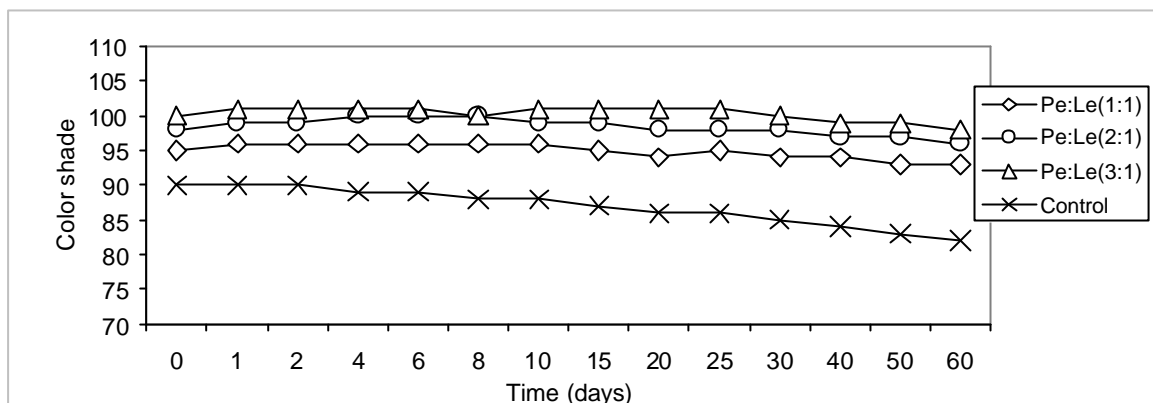


Figure (5.21) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/5% ethanol microemulsions containing curcumin during storage in darkness.

5.4.1.3 Effect of normal electric light

The changes in color shade of samples in this case were very slight where it changed from 100 to 85 as in figures 5.22 and 5.23. These figures showed also that the changes in color shade in case of control were higher than those in case of the samples. These results indicated that the color of curcumin solubilized in microemulsions stored under normal electric light changed from slight yellow to yellow and in case of control from yellow to orange.

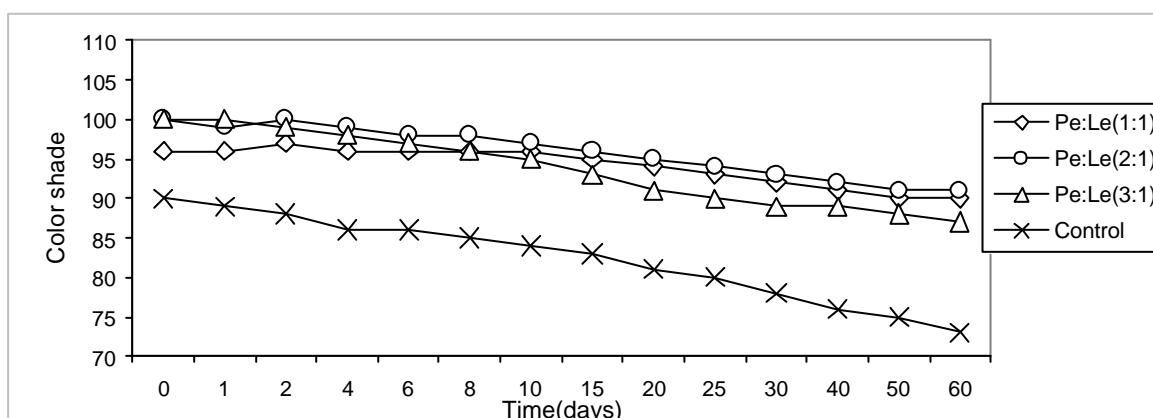


Figure (5.22) Changes in color shade of lecithin (Le)/peppermint oil (Pe) microemulsions containing curcumin during storage under normal electric light.

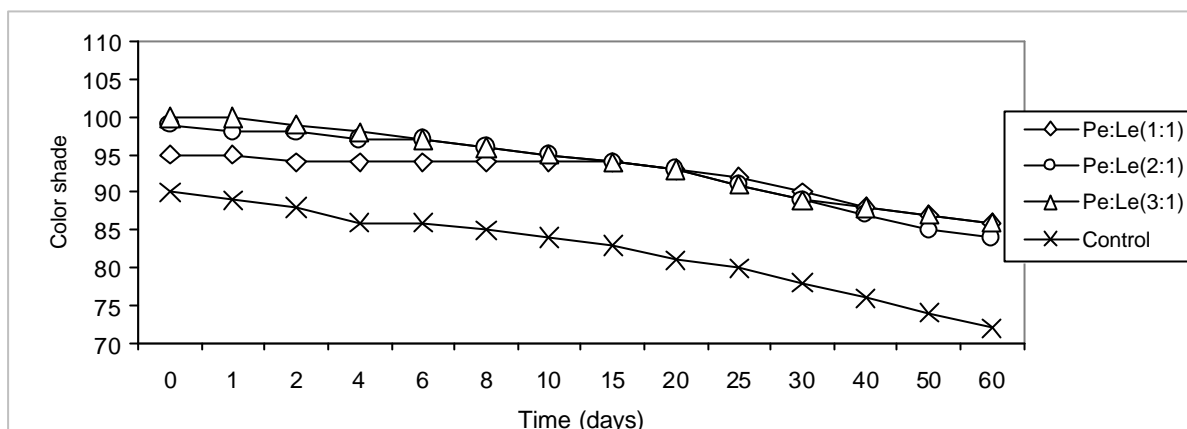


Figure (5.23) Changes in color shade lecithin (Le)/peppermint oil (Pe)/5% ethanol microemulsions and containing curcumin during storage under normal electric light.

5.4.2 Stability in lecithin/peppermint oil/soybean oil microemulsions

5.4.2.1 Effect of UV light

Alteration in color shade of samples and control stored under UV light were given in figures 5.24 and 5.25. The color shade of control changed greatly while it decreased from 90 at the beginning of storage period, to 46 after 30 days, this means the color changed from yellow to red. The change in case of microemulsions was very slight in all ratios of peppermint oil, soybean oil and lecithin..

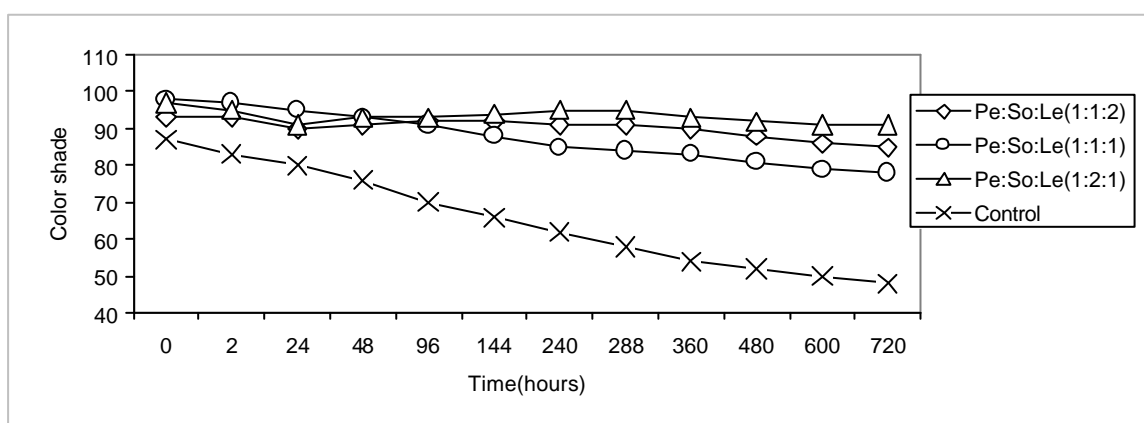


Figure (5.24) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/soybean oil (So) microemulsions containing curcumin during storage under UV light

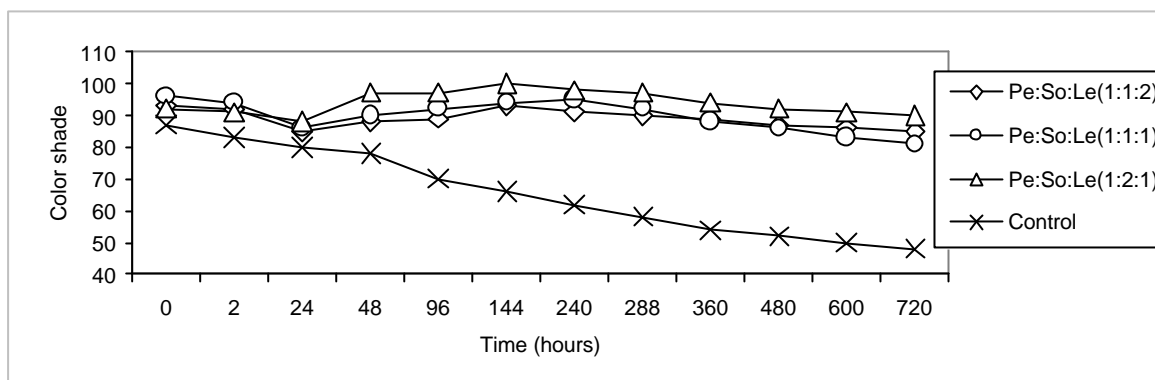


Figure (5.25) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/soybean oil (So)/5% ethanol microemulsions containing curcumin during storage under UV-light.

5.4.2.2 Effect of darkness

It should be noted from figures 5.26 and 5.27 that the color shade of samples and control stored in darkness for 60 days were somewhat stable. However, the color was remained slight yellow in case of samples at all ratios of peppermint oil, soybean oil and lecithin either without or with ethanol and yellow in case of control.

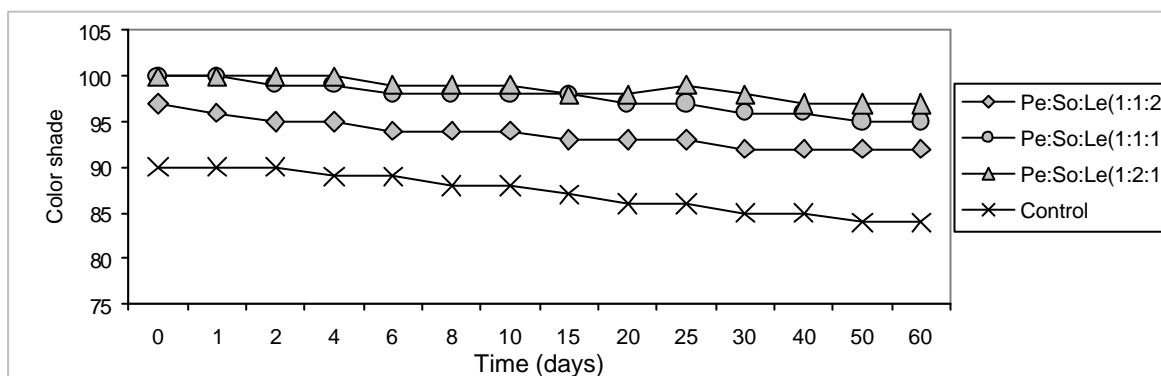


Figure (5.26) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/soybean oil(So) microemulsions containing curcumin during storage in darkness

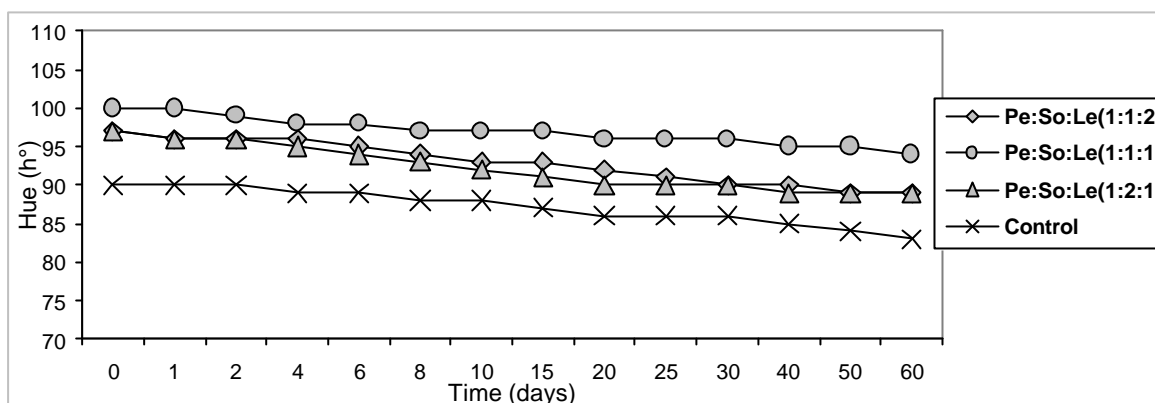


Figure (5.27) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/soybean oil (So) microemulsions containing curcumin with 5% ethanol during storage in darkness.

5.4.2.3 Effect of normal electric light

The results in figures 5.28 and 5.29 showed that the changes in color shade of samples without ethanol were lower than those with ethanol where the differences in the color shade between the zero time of storage and after 60 days were about 8 and 12 respectively. On the other hand, the changes in color shade of control were higher than those in samples where the color shade of control decreased from 90 at the zero time to 72 after 60 days of storage. These results indicated that the changes in the color of samples stored under normal electric light were slight where it changed from slight yellow to yellow but in case of control it changed from yellow to orange. In addition, the differences between the various ratios of peppermint oil, soybean oil and lecithin which used to prepare microemulsion were slight.

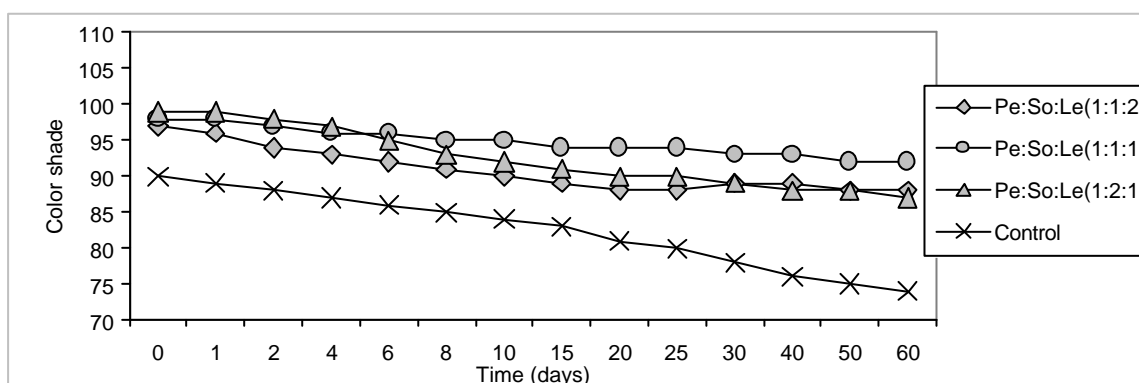


Figure (5.28) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/soybean oil (So) microemulsions containing curcumin during storage under normal electric light.

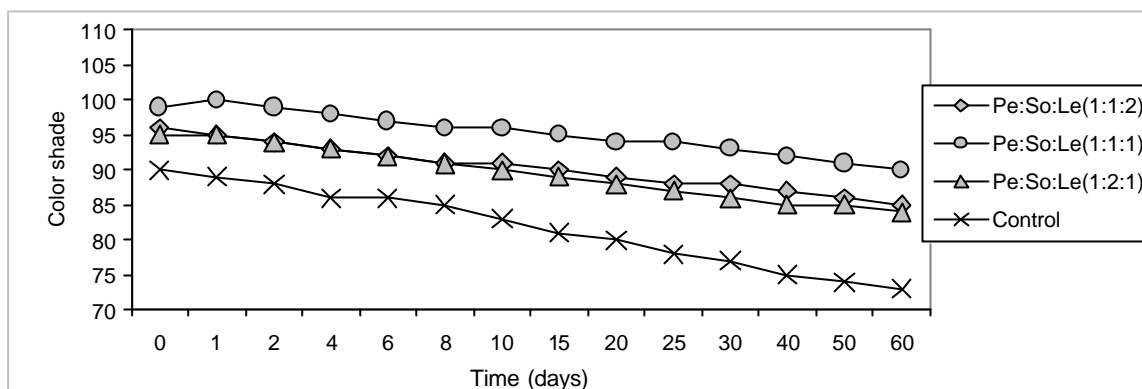


Figure (5.29) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/ soybean oil (So)/5% ethanol microemulsions containing curcumin during storage under normal electric light.

5.4.3 Stability in lecithin/peppermint oil/peanut oil microemulsions

5.4.3.1 Effect of UV light

The presented results in figures 5.30 and 5.31 showed that the changes in color shade of samples at ratio 1:2:1 of peppermint oil, peanut oil and lecithin were unremarkable either without or with ethanol, while these changes in case of ratios 1:1:2 and 1:1:1 were slight where decreased from 95 to 85. On the other hand, reversible results were found in case of control, where the color shade decreased from 90 to 46 after 30 days. Generally, it should be concluded that the color of samples was somewhat stable where it remained yellow in all cases, but the color of control changed from yellow at the beginning of storage period to red after 30 days.

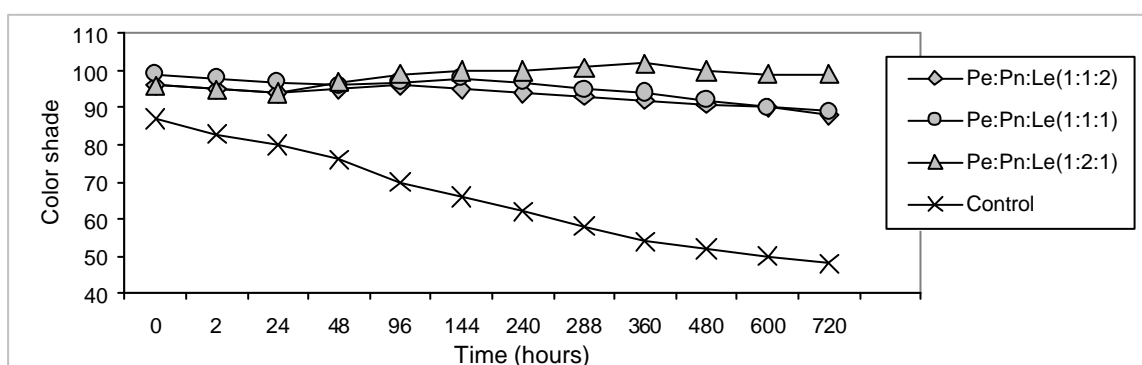


Figure (5.30) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/peanut oil (Pn) microemulsions containing curcumin during storage under UV light.

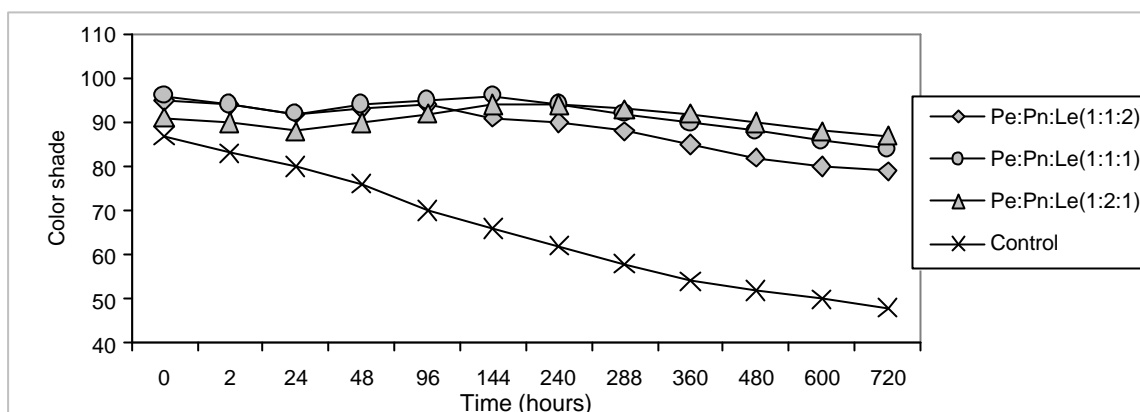


Figure (5.31) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/peanut oil (Pn)/5% ethanol microemulsions containing curcumin during storage under UV light.

5.4.3.2 Effect of the darkness

The results in figures 5.32 and 5.33 indicated that the color shade of samples stored in the darkness was stable and the color remained yellow after 60 days of storage at all ratios of peppermint oil, peanut oil and lecithin either without or with ethanol. It can be observed from the same figures that the changes in color shade of control were small where it decreased from 90 to 82 after 60 days of storage, in addition the color in this case remained yellow.

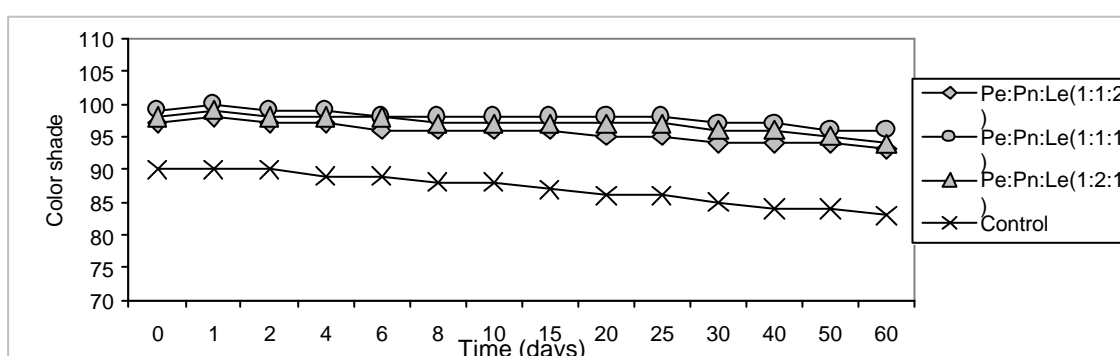


Figure (5.32) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/peanut oil (Pn) microemulsions containing curcumin during storage in darkness.

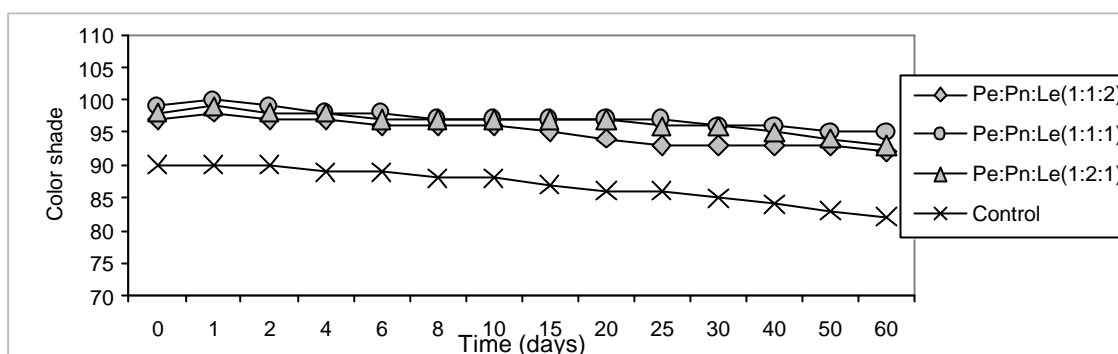


Figure (5.33) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/peanut oil (Pn)/5% ethanol microemulsions containing curcumin during storage in darkness.

5.4.3.3 Effect of normal electric light

In this case the color shade of samples either without or with ethanol at all ratios of peppermint oil, peanut oil and lecithin was somewhat stable and it of control decreased from 90 at zero time to 72 after 60 days of storage as showed in figures 5.34 and 5.35. From these results it can be said that the color of curcumin solubilized in microemulsion prepared by using peppermint oil, peanut oil, lecithin and water either without or with ethanol during the storage under normal electric light for 60 days was stable where it remained yellow while the color of control which containing curcumin solubilized in ethanol 80% and stored at the same condations changed from yellow to orange.

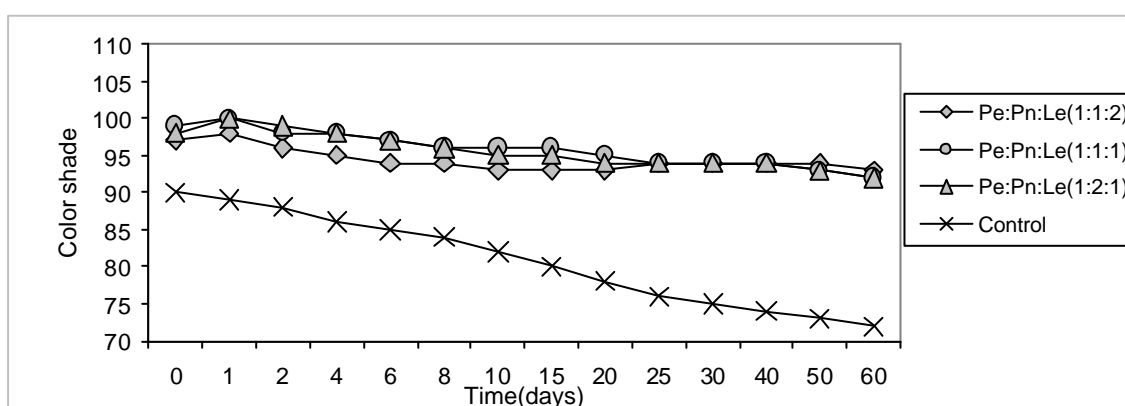


Figure (5.34) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/peanut oil (Pn) microemulsions containing curcumin during storage under normal electric light.

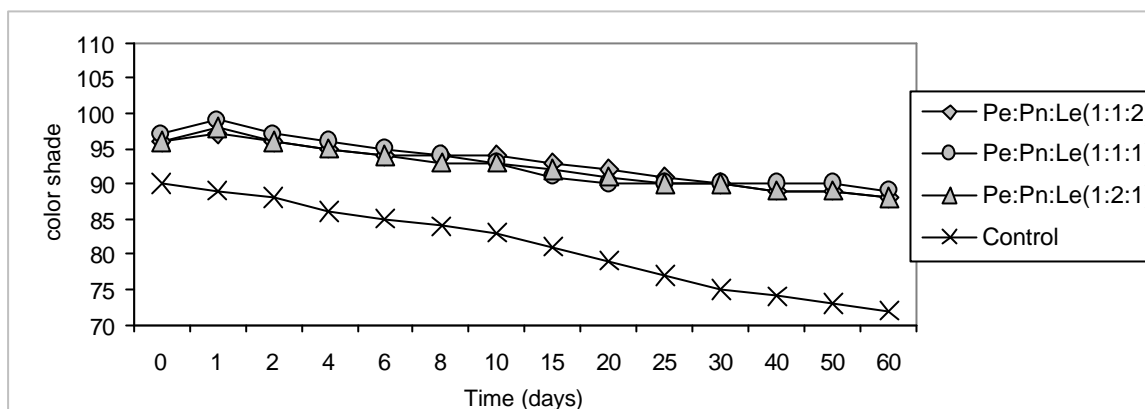


Figure (5.35) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/peanut oil (Pn)/5% ethanol microemulsions containing curcumin during storage under normal electric light.

5.4.4 Stability in lecithin/peppermint/rapeseed oil microemulsions

In this case microemulsions were prepared by using peppermint oil mixed with rapeseed oil as lipophilic phase and lecithin at ratio 1:1:2, 1:1:1 or 1:2:1. The water was added at 3% without or with 5% ethanol. Curcumin was solubilized at 0.4% in the microemulsion samples and in control which prepared by using 80% ethanol in water.

5.4.4.1 Effect of UV light

The changes in color shade of curcumin solubilized in microemulsions containing peppermint oil, rapeseed oil and lecithin at ratios 1:1:2, 1:1:1, 1:2:1 and water without or with ethanol as well as the changes in color shade of control during storage under UV light for 30 days presented in figures 5.36 and 5.37. From these figures, it should be noted that the changes in color shade of control were higher than those of samples. The color in case of control changed gradually from yellow to red while in case of samples it was approximately stable in case of ratio 1:2:1 or changed slightly from yellow to orange in other ratios. Apparent from the same figures that the changes in color shade were somewhat higher in samples which with ethanol than those without it.

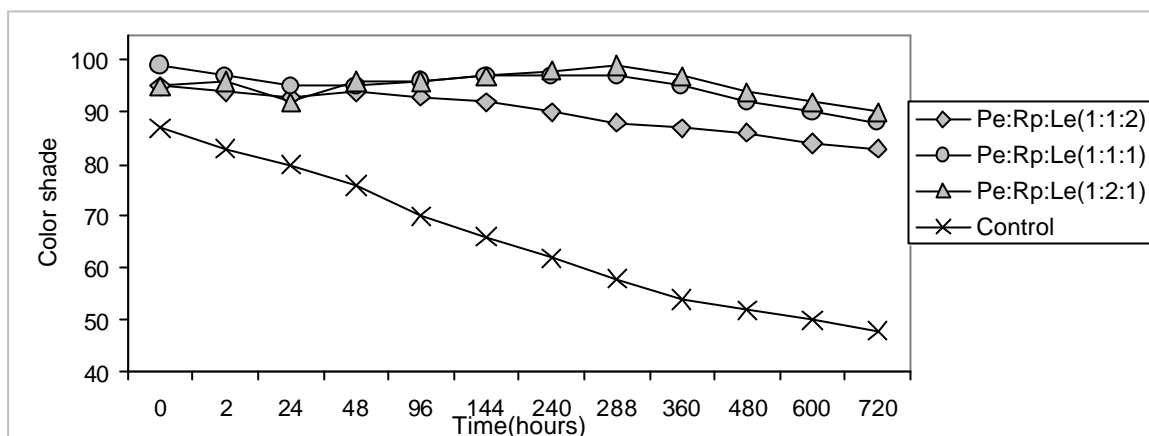


Figure (5.36) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/rapeseed oil (Rp) microemulsions containing curcumin during storage under UV light.

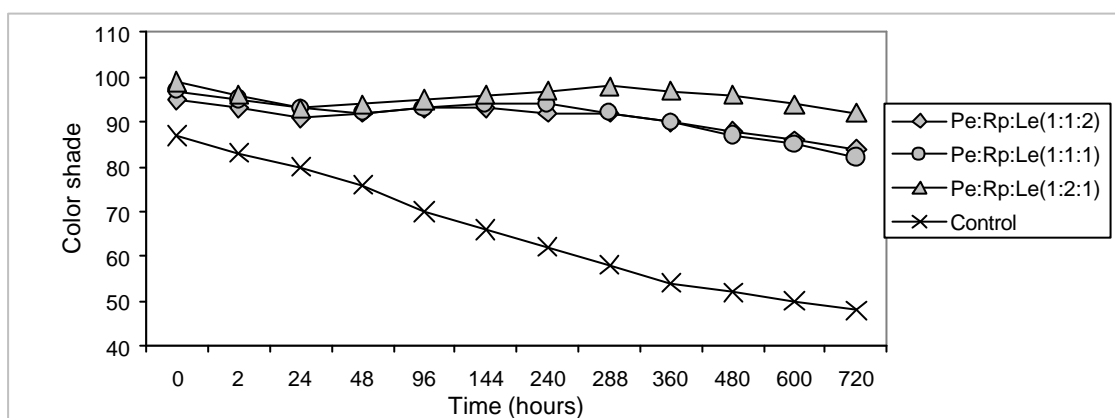


Figure (5.37) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/rapeseed oil (Rp)/5% ethanol microemulsions containing curcumin during storage under UV light.

5.4.4.2 Effect of the darkness

The color shade was stable in case of microemulsion samples and it decreased slightly in case of control where the color remained in range slight yellow (color shade 98) and yellow (color shade 90) of samples and control respectively after 60 days of storage in the darkness. The changes in color shade were similar at all ratios of peppermint oil, rapeseed oil and lecithin either without or with ethanol. These results were illustrated in figures 5.38 and 5.39.

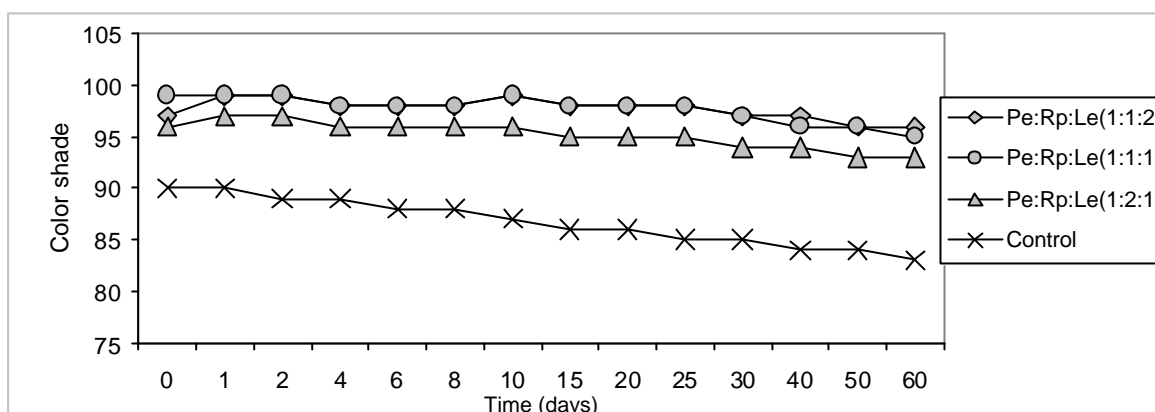


Figure (5.38) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/rapeseed oil (Rp) microemulsions containing curcumin during storage in darkness.

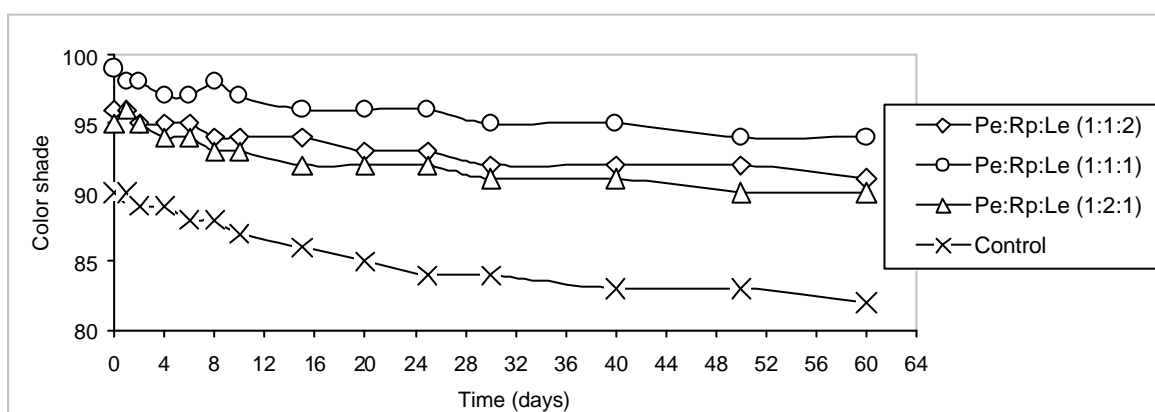


Figure (5.39) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/rapeseed oil (Rp)/5% ethanol microemulsions containing curcumin during storage in darkness.

5.4.4.3 Effect of normal electric light

It should be noted in figures 5.40 and 5.41 that the color shade of microemulsion samples was stable at all ratios either without or with ethanol after 60 days of storage under normal electric light while in case of control it decreased from 90 at the beginning of storage to 70 after 60 days. It is clear from these results that the color of microemulsion samples was stable comparing with that in case of control where it changed from yellow to orange.

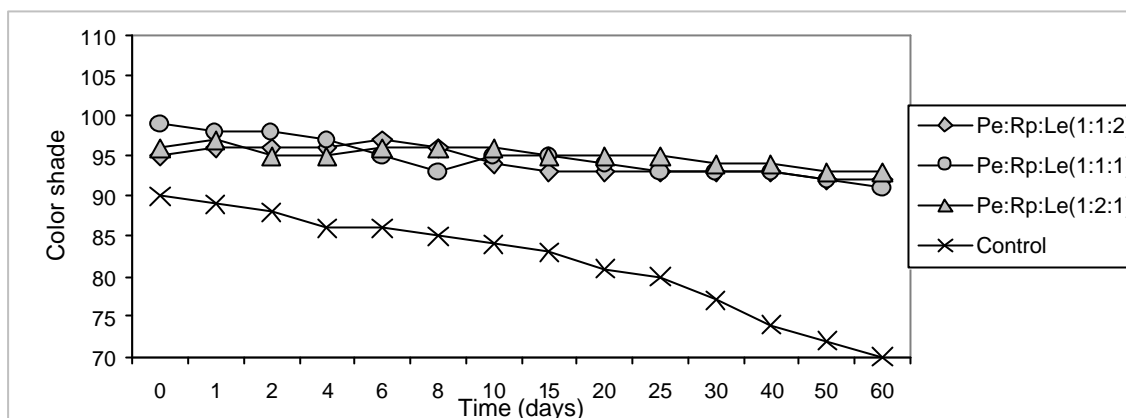


Figure (5.40) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/rapeseed oil (Rp) microemulsions containing curcumin during storage under normal electric light.

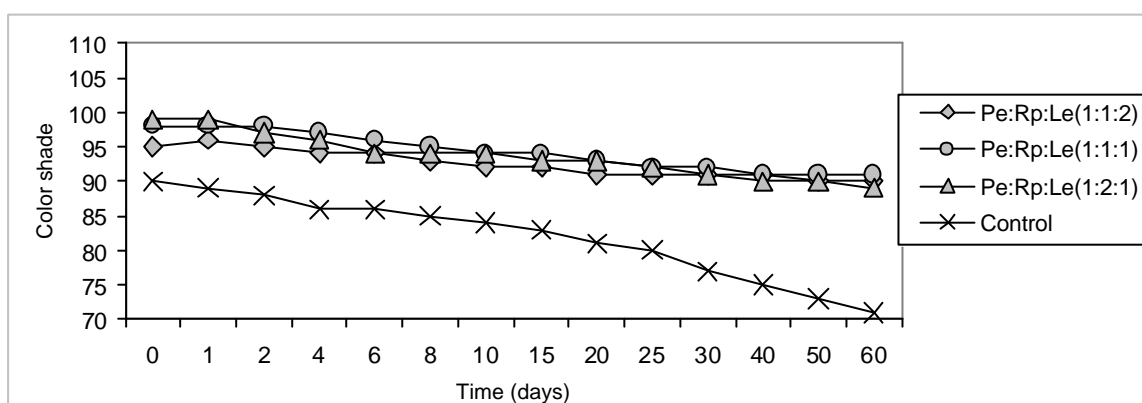


Figure (5.41) Changes in color shade of lecithin (Le)/peppermint oil (Pe)/rapeseed oil (Rp)/5% ethanol microemulsions containing curcumin during storage under normal electric light.

5.4.5 Stability in monoolein microemulsions

The monoolein microemulsions were prepared by using 3% water, 10% ethanol and monoolein either alone, mixed with soybean oil or with Tween20 at ratio 1:1. The control used in this case was 80% of ethanol in the water. Curcumin was solubilized in these microemulsions and in control at concentration 0.2%. The stability of curcumin was determined as the change of color shade occurred during the storage under UV light, in darkness or under normal electric light. The results were illustrated in figures 5.42, 5.43 and 5.44. It can be observed that the color shade of all microemulsion samples during storage either under UV light, in darkness or under electric light was stable and the color was lemon yellow at all time of storage. In case of control the color shade changed during storage under UV and normal electric light but it was approximately stable during storage in the darkness. The change of control in case of storage under UV light was higher than that under normal electric light where the color changed from yellow to red and from yellow to orange respectively.

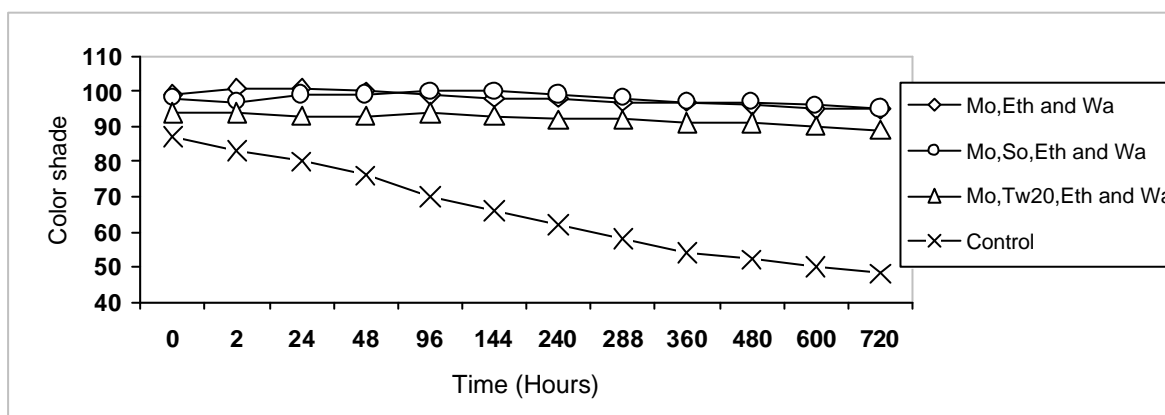


Figure (5.42) Changes in color shade of monoolein microemulsions containing curcumin during storage under UV light. Mo= monoolein, Eth=ethanol, Wa= water, Tw20=Tween20 and So=soybean oil.

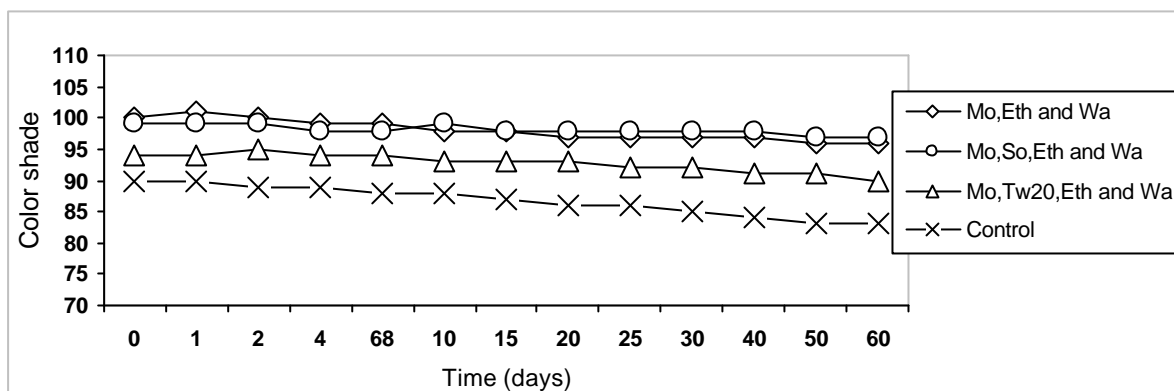


Figure (5.43) Changes in color shade of monoolein microemulsions containing curcumin during storage in darkness. Mo=monoolein, Eth=ethanol, Wa=water, Tw20=Tween20 and So=soybean oil.

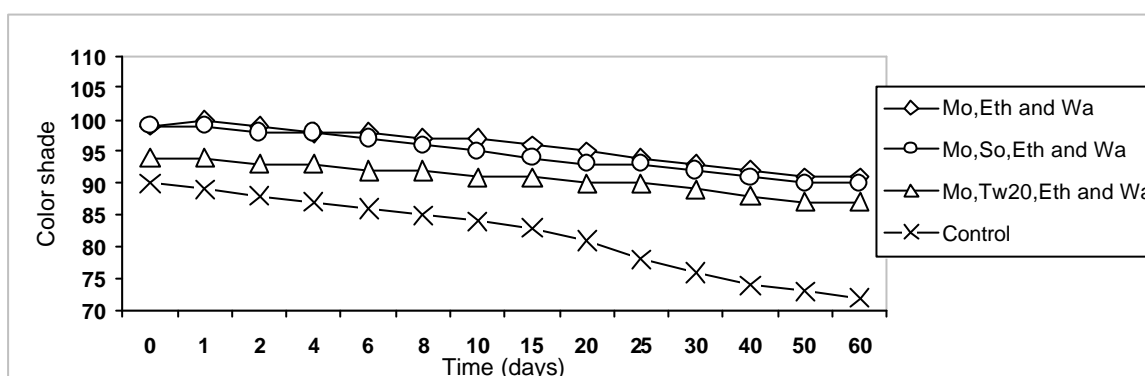


Figure (5.44) Changes in color shade of monoolein microemulsions containing curcumin during storage under normal electric light. Mo=monoolein, Eth=ethanol, Wa=water and So=soybean oil.

5.4.6. Stability in peppermint oil/Tween20 (1:1) microemulsions

The microemulsions in this case were prepared by using peppermint oil, Tween20 at ratio 1:1 and water or an aqueous solution of 20% NaCl, sucrose or citric acid. The curcumin was dissolved in these microemulsion at 0.4%(w/w). The samples were stored under UV light for 30 days, in darkness or under normal electric light for 60 days. The control (with Tween20 microemulsions) was solution of Tween20 and water at ratio 1:1.

5.4.6.1 Effect of UV light

It could be noted in figure 5.45 that the color shade of microemulsion samples containing water, sucrose or citric acid as aqueous phase was somewhat stable and the color was yellow to end of storage period but in case of microemulsion sample prepared by NaCl solution as aqueous phase the change in color shade was great where it decreased gradually from 85 at first to 65 at the end of storage period (720 hours), the color of this sample ranged between

yellow at zero time and orange brown at the end of storage. As for control the color shade decreased gradually from 77 at the beginning of storage to 52 at the end, thus the color changed from orange at first to red at the end of storage.

5.4.6.2 Effect of darkness

In this case the color shade of microemulsion samples containing water, sucrose or citric acid was stable and the color was lemon yellow in case of citric acid and yellow in other three cases as shows in figure 5.46. Likewise, in case of control the color shade was somewhat stable. On the other hand, the color shade of sample containing NaCl solution changed strongly where decreased from 95 at first to 45 after 60 days of storage, the color in this case changed from yellow to red at the end of storage.

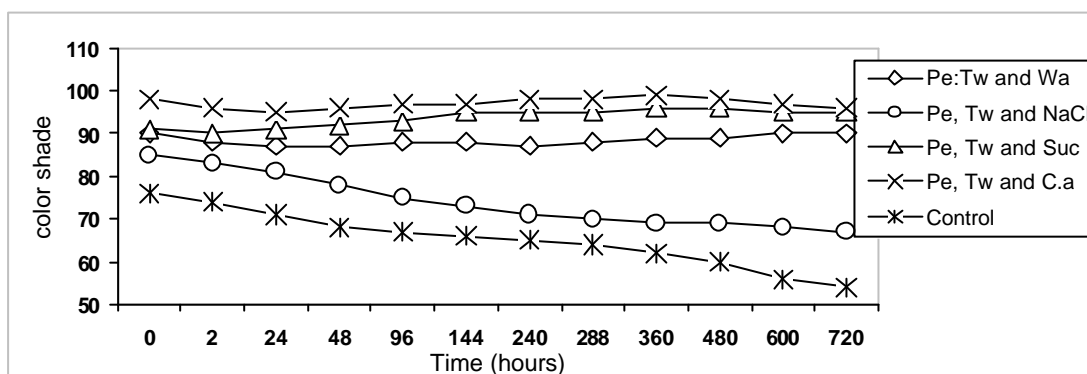


Figure (5.45) Changes in color shade of peppermint oil (Pe)/Tween20 (Tw) microemulsions containing curcumin during storage under UV light. Pe : Tw 1:1, Wa=water, NaCl= sodium chlorid, Suc=sucrose, C.a=citric acid

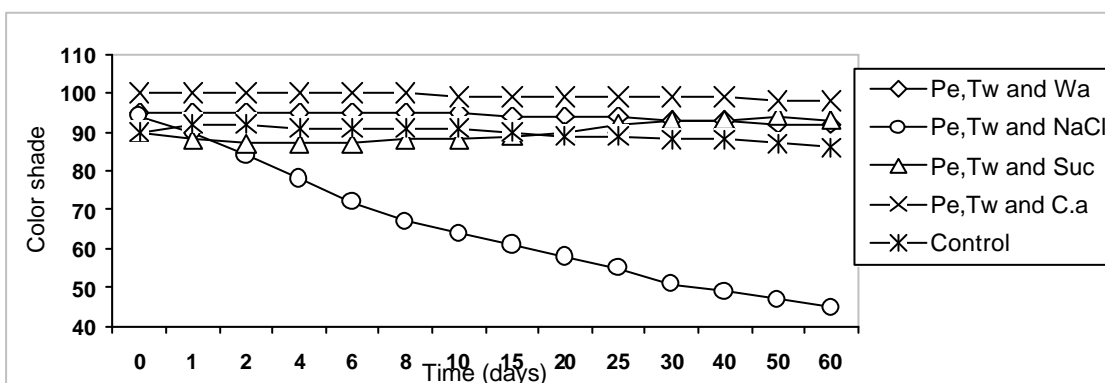


Figure (5.46) Changes in color shade of peppermint oil (Pe)/Tween20 (Tw) microemulsions containing curcumin during storage in darkness. Pe : Tw =1:1, Wa=water, NaCl=sodium chlorid, Suc= sucrose and C.a=citric acid

5.4.6.3 Effect of normal electric light

The results illustrated in figures 5.47 showed that the color shade of microemulsion samples containing water, sucrose or citric acid as aqueous phase was approximately stable, while in case of microemulsion prepared by using NaCl solution the color shade decreased strongly from 95 at the first to reached 45 after 60 days of storage. In case of control the color shade decreased gradually from 90 at the first to 60 at the end of storage.

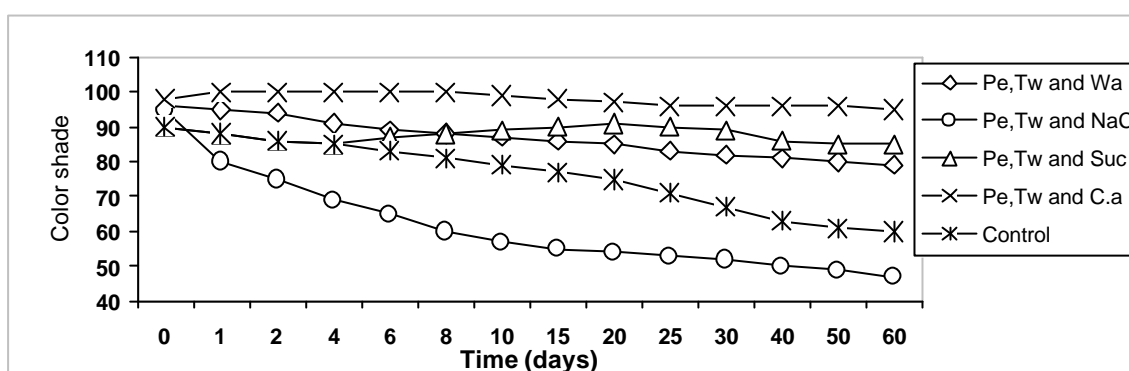


Figure (5.47) Changes in color shade of peppermint oil (Pe)/Tween20 (Tw) microemulsions containing curcumin during storage under normal electric light. Pe : Tw =1:1, Wa=water, NaCl=sodium chlorid, Suc= sucrose and C.a=citric acid

5.4.7 Stability in peppermint oil/Tween20 (1:4) microemulsions

5.4.7.1 Effect of UV light

It could be observed from figure 5.48 that the color shade of microemulsion samples was somewhat stable, where the color remained yellow in all cases during storage, but in case of control the color shade decreased gradually from 85 at zero time to 50 after 30 days of storage, thus the color in this case changed from yellow at the beginning to red at the end of storage period.

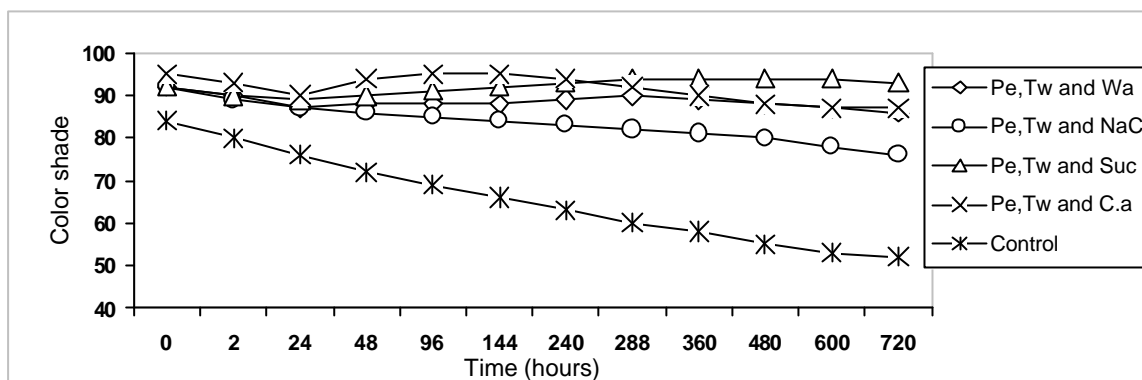


Figure (5.48) Changes in color shade of peppermint oil (Pe)/Tween20 (Tw) 1:4 microemulsions containing curcumin during storage under UV light. Wa=water, NaCl=sodium chlorid, Suc=sucrose and C.a=citric acid

5.4.7.2 Effect of darkness

In this case the color shade of microemulsion samples containing water, sucrose or citric acid was stable, where the color remained yellow to end of storage period and similar result was obtained in case of control as shows in figure 5.49. On the other hand, the color shade of microemulsion sample containing NaCl as aqueous phase decreased gradually from 90 at the beginning to 65 after 60 days of storage, thus the color in this case changed from yellow to orange.

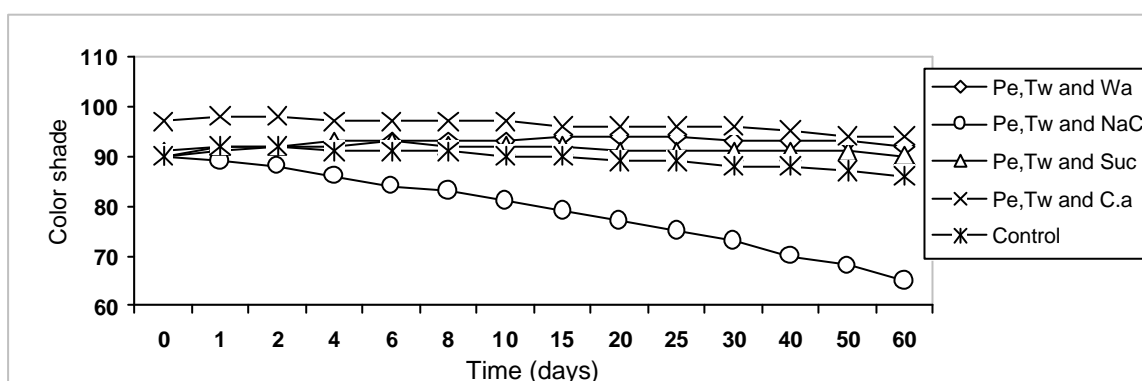


Figure (5.49) Changes in color shade of peppermint oil (Pe)/Tween20 (Tw) 1:4 microemulsions containing curcumin during storage in darkness. Wa=water, NaCl=sodium chlorid, Suc=sucrose and C.a=citric acid

5.4.7.3 Effect of normal electric light

From the results presented in figure 5.50, it should be noted that the color shade of microemulsion samples containing water, sucrose or citric acid as aqueous phase was stable during storage under normal electric light for 60 days and the color remained yellow. On the other hand the color shade in case of sample containing NaCl solution as aqueous phase and

also in case of the control the color shade decreased gradually but the change was higher in case of sample containing NaCl solution than that in case of control where the color changed from yellow to red and from yellow to orange respectively.

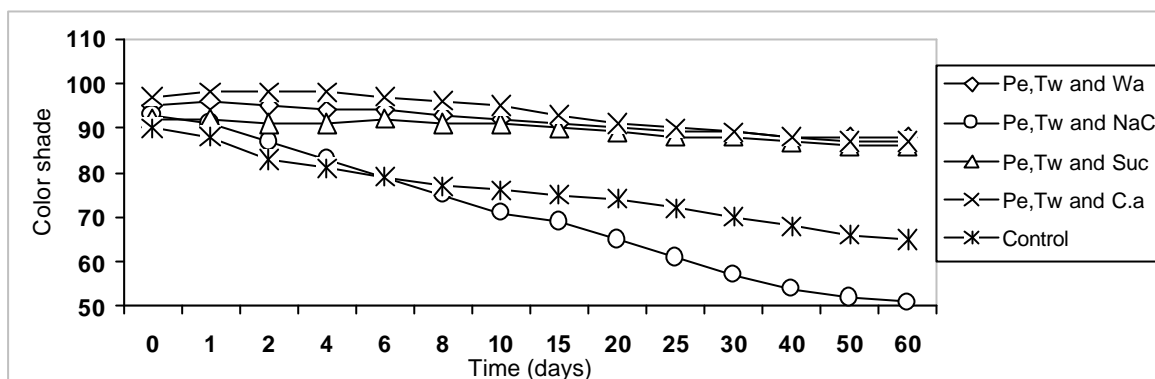


Figure (5.50) Changes in color shade of peppermint oil (Pe)/Tween20 (Tw) 1:4 microemulsions containing curcumin during storage under normal electric light. Wa=water, NaCl=sodium chlorid, Suc=sucrose and C.a=citric acid

6 Discussion

This work investigates the possibility of preparation microemulsions from suitable components for food uses. The microemulsions possess special characteristics of high solubility capacity, long term stability and ease of preparation. They also have the potential ability to solubilize both lipophilic and hydrophilic substances. Curcumin is a natural colorants and has problems for its uses direct in foods due to its insolubility in water and poor solubility in vegetable oils as well as it is sensitive to light. This work also investigates solubility and stability of curcumin when dissolved in these microemulsions. It is expected that the microemulsions due to their previous characteristics will offer good results to solubilize and stabilize curcumin against light during storage.

6.1 Preparation of microemulsions

Microemulsions containing vegetable oils could be used in food products, where there is a demand for environmentally more safety formulations [Abillon *et al.*, 1986]. When the components used in the preparation of microemulsions (surfactant, oil and cosurfactant) are safe for human consumption, they become important in such fields as foods, cosmetics and pharmaceuticals [Kunieda and shinoda 1982]. Since the first microemulsion system was described by Schulman and Hoar [1943] an extensive number of papers have been published in this area, unfortunately, most of the systems described are not suitable for human consumption either in foods or in pharmaceuticals [Von Corswant *et al.*, 1997]. Aboofazeli *et al.*, [1995] stated that most of the microemulsion systems investigated are unsuitable for human health, mainly because of the ingredients used. By far the majority of work to date has involved the use of ionic surfactants, alcohol cosurfactants and oils such as hexane and benzene. All of which are unsuitable for the purposes of pharmaceutical and food formulations. Few studies using triglycerides as the lipophilic phase in microemulsions have been published. Although oils from natural sources and their derivatives, triglycerides are considered to be harmless to the human and the environment [Busch 1992], such microemulsion systems with natural oils are but infrequently described in the literature [Alander and Warnheim 1989a]. There are very few available examples of acceptable microemulsion systems for food applications. The microemulsion system without cosurfactant, with available food surfactants and oils, and no off-taste or change in

performance is suitable for this purpose [El-Nokaly *et al.*, 1991]. The preparation of microemulsions with mineral oils, synthetic surfactants and alkanols has become a well established practice and these components are, in general, harmful. The problem arises how to prepare microemulsions with nontoxic substances [Kahlweit *et al.*, 1997]. So, the problem is then to find suitable components with physiological compatibility in order to produce microemulsions for possible applications in food products.

Various microemulsion systems were prepared by using a number of natural oils as lipophilic phase such as peppermint oil alone or mixed with one of a common edible oil such as soybean, peanut or rapeseed oil. A suitable food surfactant such as lecithin, monoolein or Tween20 was used as emulsifier. The aqueous phase was either water or an aqueous solution of 20% NaCl, sucrose or citric acid in the absence or presence of ethanol as cosurfactant. The microemulsions were defined on the basis of the surfactant type: lecithin-microemulsions, monoolein-microemulsions or Tween20-microemulsions.

6.1 1 Lecithin/peppermint oil microemulsions

Microemulsions could be produced by using peppermint oil alone as lipophilic phase, lecithin as surfactant and water without ethanol as in figure 5.1. Microemulsions could also be prepared by using these components with ethanol. It was observed that the use of high quantity of lecithin is considerably difficult to solubilize in peppermint oil, due to the formation of a high viscosity mixture. It has also observed that the solubility of a greater lecithin mass in peppermint oil needs a longer time. This problem is due to the waxy semisolid structure of soybean lecithin which used in this study. On the other hand, very small water content could be solubilized in the mixture of peppermint oil and lecithin to form microemulsion, when using a small amount of lecithin. This may be due to the insufficient surfactant content, in particular, according to Bergenstahl and Fontell [1983] soybean lecithin is both strongly hydrophobic due to long hydrocarbon chains, and strongly lipophobic due to zwitterionic polar head groups.

From the same figure (5.1) it could be noted that microemulsions were produced by using peppermint oil, lecithin and water without cosurfactant. This result is very important because the formation of microemulsions by using lecithin as surfactant, natural oils (suitable for food products) and water is one of the objectives of this work. This result may be due to the fact that, microemulsions stabilized by a nonionic surfactant can be produced without cosurfactant as reported by Aboofazeli *et al.*, [1994]. Therefore the common

cosurfactant (short chain alcohols) are not useful because of their toxicity [Friberg and Rydhang, 1971]. Cosurfactants are not easy to find in foods and their addition is not a theoretical requirement [El-Nokaly *et al.*, 1991]. Moreover, most work to date studying microemulsions has utilised oils, surfactants and cosurfactants unsuitable for pharmaceutical purposes [Aboofazeli and Lawrence 1994]. On the other hand this result is different from that is obtained by Shinoda *et al.*, [1991] where they found that lecithin will not form microemulsions without the aid of a short-chain alcohol as cosurfactant. Aboofazeli *et al.*, [1994] stated that none of studies have investigated the potential of non-alcohol cosurfactants to produce microemulsions with lecithin. Binks *et al.*, [1989] said that if lecithin containing systems are to form microemulsions over a reasonably wide range of oil and water concentrations, the effective a fairly high critical packing parameter (CPP) of lecithin needs to be reduced and a short chain cosurfactant can act to reduce the effective CPP by its incorporation into the interfacial film, in addition a short chain cosurfactant can also act to increase the fluidity of the interfacial surfactant layer, thereby reducing the tendency of lecithin to form highly rigid film.

This difference between the findings obtained in this study and those in above mentioned references may be due to the peppermint oil which used in this work has special structure which different from the structure of oils used in those studies where it contains menthol, menthyl acetate and menthone, the menthol may then act as cosurfactant.

The location and extension of a microemulsion region is generally dependent on the oil structure, this is due to differences in oil penetration into the surfactant [Monduzzi *et al.*, 1997]. Alander and Warnheim, [1989b] found that the molecular weight of the oil affected the stability of microemulsions.

El-Nokaly found that the nature and concentration of the surfactant are important to obtain a maximum solubility of water in microemulsion [El-Nokaly *et al.*, 1991]. The same result was obtained, where the maximum amount of water solubilized in the system when the preparation of microemulsions without ethanol, was higher in case of ratio 2:1 of peppermint oil and lecithin than those in case of ratio 3:1. But the decrease of the water amount in case of ratio 1:1 compared to in case of ratio 2:1 may be due to the increasing of menthol in the latter ratio (owing to the increasing of peppermint oil) which may act as cosurfactant and this increases the efficiency of lecithin to solubilize a more amount of water as shows in the same figure.

The maximum amount of water decreased in general, when using the ethanol with peppermint oil and lecithin. The maximum amount of water increased only when using a high amount of lecithin and a certain amount of ethanol (4%). This may be due to the ethanol efficiency as cosurfactant depends on the ratio of lecithin to ethanol in the mixture. Aboofazeli *et al.*, [1995] reported that the major factor influencing on the lecithin based microemulsions is the nature and mixing ratio of the cosurfactant used.

6.1.2 Lecithin/peppermint oil/edible oil microemulsions

The possibility to form microemulsions by using edible oils such as soybean, peanut or rapeseed oil was investigated in the initial experiments. Unfortunately, it was seen that using these oils to prepare microemulsion was very difficult because the solubility of lecithin in these oils needs a high amount of ethanol and this is unsuitable for food uses. In addition, water is insoluble in such mixture containing edible oils and lecithin either without or with ethanol thereby, microemulsion could not be obtained. This result is in agreement with that reported by Alander and Warnheim [1989] where they found that triglycerides, in particular long alkyl chain triglycerides such as peanut oil, are considerably more difficult to solubilize into microemulsions than hydrocarbons or alkyl esters.

Kunieda *et al.*, [1988] maintained that the edible oils such as soybean, rapeseed or sunflower oil contain long alkyl chains thereby the oil may be too bulky to penetrate the interfacial film to assist the formation of the optimum curvatures. El-Nokaly *et al.*, [1991] explained this problem on the basis that the triglycerides are semi-polar compared to hydrocarbons and the emulsifier efficiency is decreased if it is lost to the bulk and is unavailable to the interface. Friberg and Kayali [1991], stated that as pointed out earlier, liquid triglycerides do not lend themselves to microemulsion formation with the traditional technique hence, a different strategy must be employed in order to prepare a microemulsion with these compounds. The vegetable oils such as soybean and peanut oils are difficult to solubilize in microemulsions because they contain long bulky alkyl chains (mainly C16, C18 and C20) and preferentially form liquid crystalline mesophases which prevent the formation of microemulsion [Joubran *et al.*, 1994].

The peppermint oil has a strong flavor and using of it in high amount is unsuitable for taste as well as it is relatively expensive comparing with the edible oils. Attempt was carried out to make a mixture of peppermint oil and one suitable edible oil as a lipophilic phase to avoid or decrease the disadvantages of peppermint oil. The results obtained from these

experiments were recorded in figures 5.2, 5.3 and 5.4. The results indicate that the use of high amount of lecithin gives a high viscosity mixture and needs a very long time for its solubility. This problem may be due to the waxy semisolid structure of lecithin (soybean lecithin) and/or both strongly hydrophobic and lipophobic characters. The hydrophobic nature due to long hydrocarbon chains on the other hand, lipophobic nature due to zwitterionic polar head groups [Bergentahl and Fontell 1983]. The using a small amount of lecithin is difficult for the miscibility of water in the mixture containing peppermint oil and edible oil to prepare microemulsions, especially when using a high amount of triglycerides used in this case. Triglyceride-microemulsions needs to a large ratio of surfactant to water as usually form microemulsions only with a high content of hydrophobic surfactants according to the study by Mönig *et al.*, [1996].

Due to the edible oils need a high amount of surfactant to prepare microemulsions [El-Nokaly *et al.*, 1991 and Mönig *et al.*, 1996] and these oils are difficult to solubilize in microemulsions [Alander and Warnheim 1989a and Joubran *et al.*, 1994], the optimum miscible amount of water to prepare a microemulsion was somewhat lower with the case in which peppermint / soybean oil system used as a lipophilic phase than the case in which peppermint oil alone used (see figure 5.1).

The water required to obtain the microemulsion containing peppermint, soybean oil and lecithin decreased also with increasing the soybean oil and decreasing the lecithin as shows in figure 5.2. This attributed to as reported by, Kuneida *et al.*, [1988] the edible triglycerides contain long alkyl chains, therefore the oil may be too bulky to penetrate the interfacial film and/or according to El-Nokaly *et al.*, [1991] in case of triglyceride microemulsions, the emulsifier efficiency is decreased due to its inclusion in the bulk and becomes unable to the interface. Consequently, the mixture solubility capacity for water decreases with increasing the soybean oil content.

The effect of ethanol addition into the mixture containing peppermint oil, soybean oil and lecithin on the maximum amount of water required to obtain the microemulsions was investigated and the results were given also in figure 5.2. From these results shows that the required water increased with increasing the ethanol but this increment reached to a certain limit after that decreased with increasing the ethanol. When using the ethanol, it should be observed that the maximum amount of water increased with increasing the lecithin content in the mixture. The results obtained by using a mixture from peppermint mixed with soybean oil, lecithin and water without or with ethanol were illustrated in figure 5.2. It is clear from these results that the free cosurfactant microemulsions could be prepared. This

result is important, where the cosurfactants are ,in general, harmful [Kahlweit *et al.*, 1997] and usually not acceptable for taste, safety, or performance [El-Nokaly *et al.*, 1991]. Moreover, in these microemulsion systems the peppermint oil content was relatively small comparing with that in the system containing peppermint oil and lecithin without edible oil. Thus, the strong flavor of peppermint oil could be reduced. This result different from these obtained by several others [Shinoda *et al.*, 1991, Aboofazeli *et al.*, 1994, Ho, *et al.*, 1996] where they found that the use of cosurfactant (short chain alcohol) is necessary to produce lecithin based microemulsions. This can be explain on the basis that the nature of oil affect the efficiency of lecithin and/or the peppermint oil used (in this study) act as cosurfactant because it contain an alcohol (menthol).

It should be observed also that, the optimum amount of water increased by addition a small amount of ethanol after that the optimum amount of water decreased with increasing the ethanol. The results in figure 5.3 demonstrates that the microemulsions could be prepared by using water, a mixture of peppermint oil with peanut oil and lecithin without addition of cosurfactant. The microemulsions were prepared by using the same components (peppermint oil, peanut oil, lecithin and water) with ethanol as cosurfactant. The effect of ethanol on the optimum amount of water required to prepare the microemulsions was illustrated also in figure (5.3). Concerning the comparison of results obtained by using peanut oil instead of soybean oil which used in the previous experiment, it could be concluded that, the results in either cases were approximatly similar with exception of few differences. These differences included that the maximum amount of water when using soybean oil was slightly higher than that when using peanut oil especially with increasing the lecithin content (ratio 1:1:2) either without or with ethanol. The optimum amount of water when using soybean oil in case of ratio 1:1:1 needs amount of ethanol lower than that in peanut oil under the same conditions. This could be due to the differences in the chemical structure between soybean oil and peanut oil, where the first oil contains unsaturated fatty acids relatively higher than the latter oil. This explain is agreement with that repored by Parris *et al.*, [1994], where they fount that the nature of oil affect the oil miscibility in microemulsions. They found also that triglycerides containing unsaturated or short chain fatty acids had improved miscibility of oil in microemulsions compared to triglycerides with saturated or long chain fatty acids.

Microemulsions free cosurfactant were also prepared by using peppermint mixed with rapeseed oil as lipophilic phase, lecithin and water and the results were illustrated in figure 5.4. The effect of ethanol in this microemulsion system was illustrated in the same figure (5.4). It was observed that the effect of ethanol on the water miscibility in this system was to

some extent similar to the above case. The results obtained in this case (using rapeseed oil) compared to the results in case of using soybean oil (fig. 5.2) or peanut oil (fig. 5.3) indicated that the results in the three cases were almost similar with some exceptions. These exceptions included that the optimum amount of water when using rapeseed oil without ethanol was higher especially in case of ratio 1:1:1 of peppermint oil, rapeseed oil and lecithin than that when using soybean oil or peanut oil at the same conditions. On the other hand, on addition of ethanol the maximum amount of water was somewhat higher in case of using rapeseed oil than those in case of soybean or peanut oil. This is thought to be due to the difference in the chemical structure of the used edible oils. Aboofazeli *et al.*, [1994] studied partial phase diagrams of systems containing water, egg lecithin, propanol and different polar oils such as Miglyol 812 and soybean oil, and they found that the influence of the oil and the ratio of egg lecithin to propanol on the microemulsion area was remarkable.

6.1.3 Monoolein microemulsions

The monooleate and linoleate could act as cosurfactant since they are different in hydrocarbon moiety size. The oleate and linoleate groups are suitable as surfactant because of their unsaturation and its known effect in increasing the interfacial fluidity [El-Nokaly *et al.*, 1991]. The pure mono- or di-glycerides are very expensive, therefore its use in food industry is not economic. Monoolein used in this study contains mixture of mono- and di-glycerides as well as triglycerides, therefore it can be used as surfactant and oil to prepare the microemulsions. The addition of any amount of water into either monoolein alone or mixed with soybean oil could not produce a microemulsion. But when using either additional surfactant such as Tween20 or ethanol (as cosurfactant) the microemulsion could be formed as shown in figure 5.5. This means, using of monoolein either alone or mixed with soybean oil is insufficient to prepare microemulsions, this is attributed to the surfactant content (mono- and di-glycerides) present in monoolein are small and the triglyceride microemulsions need a high amount of surfactant [El-Nokaly *et al.*, 1991 and Möing *et al.*, 1996]. Friberg and Kayali [1991] reported that triglycerides do not lend directly to microemulsion formulation with the traditional technique hence, a different strategy must be employed to prepare a microemulsion with these compounds. In this experiment triglyceride microemulsions were prepared by using two surfactants (monoolein and Tween20) or with relatively high content of ethanol as cosurfactant. Troptow [1971] used two surfactants

(Tween20 and G1045) to prepare soybean oil microemulsion and found that less than 10% of oil could be dissolved in water with a 30/70 of surfactant mixture respectively.

It should be also noted that from this figure (5.5) the maximum amount of water to obtain microemulsion was higher in case of using monoolein alone than that in case of mixture of it and soybean oil. The addition of Tween20 as another emulsifier with monoolein allowed to increase the amount of water required to form the microemulsion. This indicates that the use of a high amount of surfactant has a great effect on water miscibility in monoolein microemulsions. The nature and concentration of the surfactant become of utmost importance to obtain a maximum solubility in a given water/oil microemulsion [El-Nokaly *et al.*, 1991]. Apparent also from the results in the same figure (5.5) that the water solubility increased gradually with increasing of ethanol in all cases. This may be ascribed to the effect of cosurfactant on surfactant efficiency, where the role of cosurfactant together with the surfactant is to lower the interfacial tension down to a very small even transient negative value at which the interface would expand to form fine dispersed droplets, and subsequently adsorb more surfactant and surfactant / cosurfactant until their bulk condition is depleted enough to make interfacial tension positive again, this process known as spontaneous emulsification forms the microemulsion [Kunieda *et al.*, 1988], and it is assumed that the alcohol molecules adsorb preferentially at the region of strong curvature of the interfacial film, thereby reducing its rigidity [Kahlweit *et al.*, 1995]

6.1.4 Tween20 microemulsions

Polyoxyethylene sorbitan fatty acid esters have some interesting characteristics for microemulsion formation and macro-molecules such as protein solubilization [Komives *et al.*, 1994]. Troptow [1971] prepared microemulsion by using Tween20, peppermint oil and water without cosurfactant. Ayala *et al.*, [1992] described the ability of a polyoxyethylene sorbitan fatty acid esters (Tweens)-isopropanol microemulsion in hexane to solubilize pure proteins and they found that this type of the surfactants forms stable reverse micelles which can solubilize large amounts of protein and water in hexane. According to these data, preparation of microemulsions by using Tween20 was tried. In this work Tween20 microemulsion was used to solubilize and stabilize curcumin.

Microemulsions were prepared by using peppermint oil and Tween20 at various ratios with water or 20% aqueous solution of NaCl, sucrose or citric acid. The results were illustrated in figure 5.6, showed that when using a high amount of peppermint oil (more than in ratio 1:1), the microemulsion could not formed, since water was insoluble in such mixture. This may be due to inefficiency of the emulsifier content. The optimum amount of aqueous phase much increased and linearly with increasing the Tween20 in the mixture as seen in figure 5.5. It should be also noted that the maximum amount of aqueous phase solubilized to produce Tween20-microemulsion was slightly lower only in case of NaCl relative to other cases which were approximately similar. This is possibly due to the salting out effect of NaCl, since the electrolyte lowers the solubility of nonionic surfactants in water [Brandt *et al.*, 1997]. The much and linear increase of maximum amount of aqueous phase with increasing the Tween20 content is may be due to the high hydrophilicity of Tween20. This result in agreement with that obtained by Osborne *et al.*, [1991], where they found that the surfactant concentration has a significant influence on the water solubility capacity of the microemulsion system containing dioctyl sodium sulfosuccinate-sorbitan monolaurate as surfactant.

The results obtained from these attempts showed that the use of this system (peppermint oil/Tween20/water) exhibit great ability to incorporate a high amount of water within the microemulsion system without cosurfactant.

6.2 Solubility of curcumin

Curcumin is insoluble in aqueous solutions and has poor solubility in other solvents [Henry 1996]. Microemulsions have the potential ability to solubilize both lipophilic and hydrophilic species, which allows for a variety of flavoring and coloring agents having vastly different physical properties to be dissolved within a system [Friebeg and Burasczenska 1978]. In recent years, microemulsions have been identified as potential delivery systems for lipophilic substrates due to their transparent appearance, long term stability, high solubility capacity and ease of preparation [Malmsten 1996]. This study describes attempt to formulate microemulsions containing suitable components for food applications and use them to develop solubility and stability of curcumin.

Curcumin solubility was determined on the basis of the maximum amount of curcumin solubilized in the dissolution medium without sediment. The solubility was measured as

percent of curcumin in the dissolution medium by weight. Dissolution mediums were either each solvent used to prepare microemulsions, each mixture contained the oil, surfactant and cosurfactant (if used) or each microemulsion system prepared in this study.

6.2.1 Curcumin solubility in solvents

Curcumin is not an ideal product for direct use with food products since it is insoluble in water and has poor solubility in other solvents. Thus it is usual for curcumin to be changed into a convenient application form. This is achieved by dissolving the curcumin in a mixture of food-grade solvent and accepted emulsifier [Henry 1996].

The results were recorded in table 5.1. It could be noted that the curcumin is insoluble in water and other aqueous solution of 20% NaCl, sucrose or citric acid, while the solubility was very slight in case of edible oils. On the other hand, the curcumin solubility in case of ethanol, peppermint oil or monoolein was higher than that in edible oils. The curcumin solubility in Tween20 was considerably high comparing with other solvents. This may be due to the curcumin is soluble in the emulsifiers owing to its nature, where they contain both a lipophilic and hydrophilic group.

6.2.2 Solubility of curcumin in microemulsions

The solubility of curcumin in microemulsions containing oil, surfactant and oil in absence or presence of ethanol was determined. The effect of water content in microemulsion system on the curcumin solubility was investigated and the results recorded in figures from 5.7 to 5.17.

6.2.2.1 Curcumin solubility in lecithin/peppermint oil microemulsions

The curcumin solubility in system prepared by using peppermint oil and lecithin decreased slightly after addition the water as shows in figure 5.7. This may be due to the curcumin is insoluble in water and/or the water decreases the solubility capacity of oil. From a formulation viewpoint, the increased oil content in microemulsions may provide a greater opportunity for the solubility of poorly water soluble agents [Gao *et al.*, 1998]. Comparing the results in figure 5.7 with those in figure 5.8 could be noted that the solubility increased slightly after addition of the ethanol. This is thought to be due to the ethanol act to reduce the tendency of lecithin to form a highly rigid film [Binks *et al.*, 1989], thus lowering the interfacial fluidity thereby increases the solubility capacity of the mixture. A similar result

was obtained by Gao *et al.*, [1998], where they found that the solubility of cyclosporin A increased slightly with increasing the cosurfactant when dissolving cyclosporin A in microemulsions based on triglyceride, polyoxyethylated castor oil as surfactant and Transcutol as cosurfactant and they suggested that this slight increase may be due to the excess of cosurfactant exists in the water phase and increased the solubility.

Microemulsions containing peppermint oil/lecithin/water offered a good results for curcumin solubility where the solubility increased to 2 folds or 50 folds comparing with that in peppermint oil or edible oil respectively. The effect of ethanol addition as cosurfactant on the curcumin solubility was slight. The use of ratio 2:1 of peppermint oil and lecithin exhibited better result for curcumin solubility compared to that with 1:1 or 3:1. The increasing of water content in the peppermintoil/lecithin microemulsion led to slight decreasing the solubility of curcumin.

6.2.2.2 Solubility in lecithin/peppermint and soybean oil-microemulsions

It could be observed that the solubility increased with increasing the lecithin in mixture contained peppermint and soybean oil as lipophilic phase as seen in figure 5.9. This is can be explained due to the effect of lecithin as emulsifier on the solubility of curcumin. Peng and Luisi [1990] stated that an important feature of phospholipid microemulsions in their ability to solubilize larger guest molecules. The solubility increased also with adding a few amount of water but after adding more than 2% the solubility almost decreased. The increase of solubility at the first may be due to the solubility capacity of microemulsions containing all components (required to their producing) is higher than that in case of absence one or more of essential component [Gao *et al.*, 1998]. But the decreasing after addition more than 2% of water may be ascribed to the excess of water decreases the lecithin efficiency, in addition the curcumin is insoluble in water. On the other hand, the solubility increased slightly when addition the ethanol into the system contained lecithin, peppermint mixed with soybean oil and water (figure 5.10) compared to the same system without ethanol. This is possible due to the effect of ethanol on the mixture interfacial fluidity. From the same figure (5.10) apparent also the solubility decreased when using ethanol with a high amount of water content. This may be due to the excess of water incorporates with ethanol and decreases its efficiency.

6.2.2.3 Solubility in lecithin/peppermint and peanut oil-microemulsions

It could be noted that the solubility increased after addition a few amount of water into the mixture contained a lower content of lecithin and decreased with increasing the water as shows in figure 5.11. The increment (at the first) may be due to the microemulsions possess high solubilization capacity compared to many other solutions [Friberg and Burasczenska, 1978; El-Nokaly *et al.*, 1991 and Von Corswant *et al.*, 1997] owing to extremely small of their size droplets [Goa *et al.*, 1998]. The decreasing of solubility with increasing the water content than 3% may be ascribed to the curcumin insolubility in water.

The curcumin solubility in the mixture containing a high content of lecithin decreased slightly with addition a few amount of water and no more increase with increasing the water. This may be due to the water decreases the lecithin efficiency on the curcumin solubility because a part of lecithin dissolve in the water, but the no change in curcumin solubility occurred with increasing the water can be explain on the basis, the water although decreases the lecithin efficiency, leads to lowering the viscosity thereby increases the mixture solubility.

The solubility of curcumin decreased slightly when using ethanol with increasing the water in mixtures containing a smaller content of lecithin as to be seen in figure 5.12. This decrease may be due to the curcumin is insoluble in water and/or the excess of water incorporate with a part of ethanol. In contrast, in case of using a higher content of lecithin and a few amount of water, the solubility of curcumin increased when using ethanol. This is thought to be the ethanol increases the mixture fluidity consequently, the solubility capacity increased. With increasing the water, the solubility decreased slightly in the same mixture, this may be due to a part of ethanol dissolve in the excess water.

6.2.2.4 Solubility in lecithin/peppermint and rapeseed oil-microemulsions

The solubility of curcumin decreased slightly with increasing the water in case of the system containing a smaller lecithin content, this may be attributed to the combination of water with lecithin, so decreases its efficiency for solubility of curcumin. In case of the system containing a higher lecithin and a lower rapeseed oil, the solubility increased slightly after addition the water and this increase continued with increasing the water as to be seen in figure 5.13. This is thought attributed to decreasing the mixture viscosity thereat increased curcumin solubility.

The curcumin solubility increased slightly when using the ethanol as cosurfactant with peppermint and rapeseed oil/lecithin microemulsion as to be seen in figure 5.14. This may be ascribed to that the ethanol increases the mixture fluidity. Kahlweit *et al.*, [1995] stated that alkanols adsorb preferentially at the region of strong curvature of interfacial layer, thereby lowering its rigidity. As shows in the same figure (5.14) the solubility decreased slightly with increasing the water, this may be due to a part of ethanol dissolved in the excess water, thereat decreased the available amount of ethanol.

The curcumin solubility in edible oil microemulsion was more better than in edible oil itself, where the solubility increased from 0.02% in edible oil to 0.5% in case of edible oil microemulsion. The curcumin solubility was however, lower when using the edible oil with peppermint oil to prepare microemulsions than that when using peppermint oil without edible oil. The curcumin solubility decreased with increasing the edible oil content in peppermint-edible oil microemulsion, but in case of use a higher content of peanut oil the solubility of curcumin increased slightly. The increase of water content in microemulsions containing peppermint oil mixed with edible oil has slightly effect on the curcumin solubility. Likewise, the effect of ethanol addition as cosurfactant on the curcumin solubility was unremarkable.

6.2.2.5 Curcumin solubility in monoolein microemulsions

The solubility of curcumin in monoolein microemulsions containing ethanol, and water was higher than that in monoolein singly. While the solubility of curcumin in monoolein microemulsion containing soybean oil, ethanol and water was similar with that in monoolein alone as shows in figure 5.15. This indicates that microemulsions possess high solubility capacity [Malmsten 1996] due to their ultra low interfacial tension and extremely small size droplets. As reported by Gao *et al.*, [1998] the solubility of cyclosporin A (poorly soluble in the water) in a system containing all components for producing microemulsions increased markedly compared with those of system without surfactant or cosurfactant. It should be also noted that the solubility of curcumin increased strongly when using Tween20 as additional surfactant with monoolein to prepare microemulsions comparing with that in other monoolein microemulsions as shows in the same figure (5.15). This is thought to be due to the effect of Tween20 as emulsifier on the mixture solubility capacity.

Ayala *et al.*, [1992] described the ability of a polyoxyethylene sorbitan triolate (Tween85) - isopropanol microemulsion in hexane to solubilize pure proteins and they found that the

nonionic surfactant Tween85 forms stable reverse micelles in hexane which can solubilize large amounts of protein and water in hexane. It could be noted from the same figure (5.15) that the solubility in monoolein microemulsions contained soybean oil was the lowest one, this indicates that the curcumin is poor soluble in a long chain triglycerides. It could be shown that the solubility of curcumin decreased slightly with increasing the water content due to insolubility curcumin in water. The previous results showed that, the solubility of curcumin in lecithin microemulsion was higher than that in monoolein microemulsions (with exception of monoolein microemulsion containing Tween20). The use of Tween20 with monoolein to prepare microemulsion enhanced the solubilization capacity water and curcumin in microemulsions.

6.2.2.6 Curcumin solubility in Tween20 microemulsions

The curcumin solubility was determined in Tween20 microemulsions and the results were illustrated in figures 5.16 and 5.17. From these results apparent that the solubility in Tween20 microemulsions was considerably high compared to that in lecithin- or monoolein-microemulsions. This indicates that Tween20 has a high ability to solubilize the curcumin, this is due to its structure where it contain polyoxyethylene which may have high ability for solubility of macro-molecules [Komives *et al.*, 1994].

The curcumin solubility in the system containing a high content of Tween was higher than that in the system containing a smaller content, indicated that the Tween content has a great influence on the curcumin solubility. Apparent also from the results in figure 5.16 that the solubility in case of microemulsion containing citric acid as aqueous phase was the highest one. The slight increment of solubility when using citric acid is thought attributed to its effect on the interfacial tension of system. The effect of water addition on the curcumin solubility is dependent on the content of Tween, where the solubility of curcumin decreased slightly with increasing the aqueous phase in the system containing a smaller content of Tween (figure 5.16). The solubility of curcumin decreased considerably with increasing the aqueous phase when producing microemulsion with higher Tween20 content as shows in figure 5.17. This may be due to the decrease of oil content in such system. As reported by Gao *et al.*, [1998] from a formulation viewpoint, the increased oil content in microemulsions may offer a greater opportunity for the solubilization of poorly water-soluble agents.

6.3 Light stability of curcumin in microemulsions

Safety of food colorants has been a very controversial topic in recent years but most of the criticism has been usually towards the synthetic colorants. The natural colorants have been relatively few of criticism may be due to the belief that most are derived from well known food sources that have been consumed for many years [Francis 1996]. Curcumin is an interesting molecule because of the variety of biological effects, it possesses in addition to its potent anticancer activity [Khar *et al.*, 1999]. The advantages of using natural colorants are that they are generally more widely permitted in food-stuffs than synthetic colorants and, contrary to many reports, natural sources can provide a comprehensive range of attractive colors for use in the modern food industry [Henry 1996]. The problem with natural colorants has been their lack of stability for the light and oxygen, which limits its use [Lauro 1998]. Curcumin changes in the light and its sensibility to light is a factor that usually limits its use in foods [Henry 1996].

Microemulsions possess long term stability [Friberg and Burasczenska 1978, Bourrel and Schechter 1988, and Malmsten 1996]. So, the attempt for curcumin stability against light by using microemulsions was studied. Microemulsions containing curcumin were stored under UV light for 30 days, in darkness or under normal electric light for 60 days to investigate the stability of curcumin solubilized in microemulsions against light. The stability was measured as the change in color shade during the storage.

6.3.1 Curcumin stability in lecithin/peppermint oil microemulsions

The curcumin color shade was measured in the samples and control before and during storage. The results were illustrated in figures from 5.18 to 5.23. From these results, it should be noted that the color shade was stable in all samples stored either under UV light, in darkness or under normal electric light, but it decreased markedly in case of control particularly, during storage under the light. The illustrated results indicated that the microemulsions increased the stability of curcumin against light. This attributed to the infinite stability of microemulsions [Bourrel and Schechter 1988], microemulsions are thermodynamically stable because the surface free energy of them has two components stretching (positive contribution) and bending (negative contribution) the two cancel each other and the total surface free energy is extremely small about 10^{-3} mN/m, and the interaction with light is limited [Friberg and Kayali 1991]. It should be also noted that the

concentration of lecithin and oil in microemulsion system has no effect on the stability of curcumin, further the effect of alcohol is not obviously. Ho *et al.*, [1996] studied the stability of insulin in microemulsions prepared by using polyglycerol fatty acid esters as surfactant combined with short chain alcohols and they found that the stability of insulin in these systems was quite acceptable, and the influence of alcohols on the stability of insulin in microemulsions seems to be somewhat different, but not so obviously.

6.3.2 Curcumin stability in lecithin/peppermint/edible oil microemulsions

The change in the color shade of curcumin solubilized in microemulsions containing soybean oil was illustrated in figures from 5.24 to 5.29. The results for lecithin/peppermint and peanut oil microemulsions were illustrated in figures from 5.30 to 5.35. The results for lecithin/peppermint and rapeseed oil microemulsions were illustrated in figures 5.36 to 5.41.

From these results, it could be observed that no changes in the curcumin color shade in case of microemulsion samples comparing with those in control where the changes in control were considerable during storage under UV or normal electric light. It was clear that microemulsions exhibit a greater opportunity for the stability of curcumin against light. Apparent also from these results that the nature and concentration of oil and lecithin in microemulsion systems have no great effect on the stability of curcumin. Likewise, the effect of ethanol as cosurfactant in the microemulsions on the curcumin stability is not obvious. Although the curcumin is sensitive to light as reported by [Henry 1996], the microemulsions offered very good results for its stability against light for long period.

Friberg and Burasczenska [1978] reported that the advantages of a microemulsions over emulsions, suspensions, or solutions are improved stability and solubility characteristics. Since microemulsions are thermodynamically stable, separation problems that frequently occur with emulsions are avoided. Malmsten [1996] stated that, in recent years, microemulsions have been identified as potential delivery systems for lipophilic agents due to their transparent or translucent appearance, stability for long time, large solubility capacity and ease of preparation.

6.3.3 Curcumin stability in monoolein microemulsions

The results for stability of curcumin solubilized in monoolein microemulsions were recorded in figures 5.42, 5.43 and 5.44. The results shows that the color shade was stable in case of samples of monoolein microemulsions containing curcumin under UV light, in darkness or

under normal electric light. The change in color shade of curcumin solubilized in control was considerable during storage under light, and it was slight during storage in darkness. This indicates that the monoolein microemulsions give a good stability for curcumin against light for long period. The sensibility of curcumin to light is the one of the factors that usually to be limited its application in foods [Henry 1996]. Monoglycerides are nonionic surfactants widely used in the food and pharmaceutical fields [Holmberg and Österberg 1988], further some data have shown that nonionic surfactants exhibit the advantage of a high chemical stability [Hofland *et al.*, 1994].

6.3.4 Curcumin stability in Tween20 microemulsions

The results for the stability of curcumin in Tween20 microemulsions were recorded in figures from 5.45 to 5.50. It could be showed that, all Tween20 microemulsion samples exhibited a quite acceptable results for stability of curcumin against light for long period comparing with that in control, except the samples in which prepared by using NaCl as aqueous phase. Gallarate *et al.*, [1999] found that the rate of ascorbic acid oxidation, as its degradation rate, was slower in microemulsions than that in aqueous solutions. In case of Tween20-microemulsions prepared with NaCl as aqueous phase the color shade changed markedly from yellow to red. This result is thought due to the effect of sodium cation in NaCl solution on the curcumin color shade. Henry [1996] reported that, for curcumin, in general, cations will tend to induce a more orange brown color shade.

The change in color shade of samples containing NaCl was higher during storage in darkness or under normal electric light than that under UV light. This may be due to the UV light has effect on the ionization process of NaCl and/or it has effect on the efficiency of sodium cation on curcumin color shade. It should be noted that the curcumin color shade tend to be lemon yellow with low pH value and orange with high pH value. This result in agreement with that reported in Food Advisory Committee, FdAC/REP/4, HMSO, London, [1987].

It could be also observed that the influence of pH value, concentration of oil and surfactant, nature of oil and surfactant, presence of alcohol as cosurfactant in microemulsions on the stability of curcumin during storage was no remarkable.

Carlotte *et al.*, [1995] studied the rate of oxidation of linolic acid and ethyl linoleate in O/W microemulsions and the results showed that the structure of the interface in the microemulsions was especially significant to protect the systems from auto-oxidation, while

the nature of the emulsifier does not seem to greatly affect the oxidation of unsaturated molecules. Ho *et al.*, [1996] found that the microemulsions maintained similar viscosity during storage at room temperature for long period (several months), indicating that the system was thermodynamically stable. In contrast, the viscosities of macroemulsions were variable. Further, several microemulsion systems were demonstrated to be promising for oral delivery of insulin based on the results of stability experiments and acid-protection efficiency.

They found also that the influence of alcohols as cosurfactant on the stability of insulin in microemulsions seems to be not so obviously.

6.4 Conclusion

Several microemulsion systems were prepared by using different natural oils such as peppermint oil alone or mixed with a common edible oils (soybean, peanut or rapeseed oil), different surfactants such as lecithin, monoolein or Tween20 and an aqueous solution (water, 20% solution of NaCl, sucrose or citric acid) without or with ethanol as cosurfactant. The solubility of curcumin in these microemulsion systems was investigated. The stability of curcumin solubilized in these microemulsions against light was also studied during storage under UV-light for 30 days, in darkness and under normal electric light for 60 days. The stability of curcumin was determined as the change in curcumin color shade by using Measuring Color Instrument.

The obtained results can be concluded as follows :

- 1- Microemulsions could be prepared by using peppermint oil, lecithin and water without cosurfactant
- 2- The optimum amount of water required to prepare microemulsions in this case, was somewhat remarkable.
- 3- Microemulsions were also prepared by using these components with ethanol and the optimum amount of water in this case decreased slightly with increasing the ethanol.
- 4- Microemulsions could not be prepared by using edible oils as lipophilic phase and lecithin either without or with ethanol, but the use of peppermint oil with edible oil was necessary.
- 5- The optimum amount of water to obtain microemulsions by the use of edible oil and peppermint oil as lipophilic phase was lower than that in case of use peppermint oil singly.

- 6- The optimum amount of water almost decreased with increasing the edible oil content in the mixture.
- 7- When addition the ethanol as cosurfactant into the system containing peppermint oil, edible oil and lecithin, it was observed that the the optimum amount of water increased at the first with increasing the ethanol to reached a certain amount after that decreased with increasing the ethanol.
- 8- The efficiency of ethanol as cosurfactant on the water solubility in the microemulsion system depended on the lecithin content, where its efficiency increase with increasing the lecithin content.
- 9- No wide differences were noted between the edible oils used, where the nature of edible oil does not seems to greatly affect the solubility of water in the microemulsion system.
- 10- The preparation of microemulsions by using monoolein alone as surfactant could not be achieved.
- 11- The addition of ethanol or other emulsifier such as Tween20 with monoolein was important to solubilize the water in the system containing monoolein either alone or mixed with an edible oil.
- 12- Using Tween20 with monoolein has greatly affect on the solubilization of water in the mixture to prepare microemulsion, where the maximum amount of water miscible with the system was considerably remarkable compared to that when using ethanol with monoolein either alone or mixed with soybean oil.
- 13- The use of soybean oil with monoolein decreases the water solubilized in the system.
- 14- The miscibility of water increased linearly with increasing the ethanol either in case of monoolein alone, mixed with soybean oil or mixed with Tween20.
- 15- Microemulsions were prepared by using peppermint oil, Tween20 and water or an aqueous solution 20% of NaCl, sucrose or citric acid without cosurfactant.
- 16- The solubility of aqueous phase increased with increasing the Tween20 content.
- 17- NaCl seems to have the lowest solubility compared among all the tested aqueous solution, while the other aqueous solutions given similar results.
- 18- Generally, the solubilization capacity for aqueous phase in case of using Tween20 as surfactant was higher than that in case of using lecithin or monoolein.
- 19- An attempt to emulsify edible oils with Tween20 failed, where the mixture of Tween20 and edible oil was turbid and the turbidity increased after addition the aqueous phase.
- 20- The results obtained for solubility of curcumin in microemulsions showed that the solubility of curcumin in microemulsionss was greatly higher than that in edible oil.

- 21-** Solubility of curcumin in microemulsions containing peppermint oil as lipophilic phase was markedly higher than that in peppermint oil. Curcumin solubility in lecithin microemulsions containing edible oil increased slightly after addition a few amount of water, in contrast, in case of lecithin microemulsions containing peppermint oil. Solubility of curcumin in lecithin microemulsions produced by using peppermint oil was higher than that in lecithin microemulsions produced by using peppermint and edible oil.
- 22-** Solubility of curcumin in Tween20-microemulsions was considerably higher than that in lecithin or monoolein-microemulsions. The solubility of curcumin in Tween20-microemulsions increased with increasing the Tween content, but decreased with increasing the aqueous phase.
- 23-** The light stability of curcumin in microemulsions was studied as the change in the color shade during storage and the results indicated that, with exception the microemulsions prepared by using NaCl as aqueous phase, all microemulsion formulations exhibited a very good results for the stability of curcumin against either UV or normal electric light for long period. In case of microemulsions formed by using NaCl as aqueous phase the change in color shade during storage was remarkable either under light or in darkness.
- 24-** The influence of ethanol which added as cosurfactant on the stability seems to be not obviously, further pH value has no affect on the stability. The nature and concentration of oil or surfactant used to formulate the microemulsions have also no effect on the stability of curcumin.

Finally, Microemulsions could be prepared by using a suitable components for food applications. These microemulsions could be used to improve the solubility of curcumin and other natural food colorants which are insoluble in water and poorly soluble in vegetable oils. These microemulsions were demonstrated to be promising for stability of curcumin and other natural food colorants which are sensitive to light.

7 Summary

Several microemulsion formulations were prepared by using various vegetable oils such as peppermint oil alone or mixed with a common edible oils (soybean, peanut or rapeseed oil), different surfactants such as lecithin, monoolein or Tween20 and an aqueous solution (water or aqueous solution of 20% NaCl, sucrose or citric acid) without or with ethanol as cosurfactant. The solubility of curcumin in these microemulsion systems was investigated. The stability of curcumin dissolved in these microemulsions against light was also studied during storage under UV-light for 30 days, in darkness and under normal electric light for 60 days.

The obtained results indicated that microemulsions could be prepared by using peppermint oil, lecithin and water without cosurfactant. Microemulsions were also prepared by using these components with ethanol as cosurfactant. Microemulsions could not be prepared by using edible oils as lipophilic phase and lecithin either without or with ethanol, but the use of peppermint oil with edible oil was necessary. The optimum amount of water required to form microemulsions by the use of edible oil and peppermint oil was lower than that in case of use peppermint oil singly.

The preparation of microemulsions by using monoolein alone as surfactant could not be achieved. The addition of ethanol or other emulsifier such as Tween20 with monoolein was important to solubilize the water in the system containing monoolein either alone or mixed with an edible oil. The use of Tween20 with monoolein has greatly affect on the mixture solubilization capacity for water. The miscibility of water increased linearly with increasing the ethanol either in case of monoolein alone, mixed with soybean oil or mixed with Tween20.

Microemulsions were prepared by using peppermint oil, Tween20 and water or an aqueous solution of 20% NaCl, sucrose or citric acid without cosurfactant. An attempt to emulsify edible oils with Tween20 failed. The solubility of aqueous phase increased with increasing the Tween20 content. Generally, the solubilization capacity for aqueous phase in case of using Tween20 as surfactant was higher than that in case of using lecithin or monoolein.

The results obtained for solubility of curcumin showed that the solubility of curcumin in microemulsions was greatly higher than that in edible oil. Solubility of curcumin in microemulsions containing peppermint oil alone as lipophilic phase was markedly higher than that in peppermint oil. The effect of ethanol addition into lecithin microemulsions on

the solubility of curcumin was small. Solubility of curcumin in Tween20-microemulsions was considerably higher than that in lecithin or monoolein-microemulsions.

The light stability of curcumin in microemulsions was studied as the change in the color shade during storage. The results indicated that microemulsions exhibited very good results for the stability of curcumin against either UV or normal electric light for long period except the microemulsion prepared by using NaCl as aqueous phase, where the change in color shade of this microemulsion was great.

The influence of ethanol which added as cosurfactant on the curcumin stability seems to be not obviously, further pH value has no effect on the stability. The nature and concentration of oil or surfactant used to formulate the microemulsions have also no effect on the stability of curcumin.

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1975-1977	Middle School
1978-1981	Secondary School
1982-1986	Faculty of Agriculture, Department of Food Technology, Tanta University, Kafr El-Sheikh, Egypt <u>Degree:</u> B. Sc. In Agriculture Science
1987-1993	<u>Position:</u> Demonstrator in Dept. Of Food Technol., Fac. Of Agric., Tanta Uni., Kafr El-Sheikh, Egept <u>Degree:</u> Magister in Agric. Sci. (Food Technol.)
1994-1997	<u>Position:</u> Assistant Lecturer in Fac. Of Agric., Tanta Uni., Kafr El-Sheikh Egypt
Since 04/1997	Ph. D. Scholarship of Egyptian Government, Institute of Food Technology, University of Bonn, Germany