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Thesis Approval

Formulation and Characterization of Microemulsions for
Biomedical Applications




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Abstract:

Microemulsions are transparent colloidal assemblies having polar and nonpolar microdomains. They consist either of water droplets in oil or oil droplets in water where the droplets are surrounded by surfactant film, or they have a bicontinuous microstructure, i.e. continuous channels of oil and water separated by the surfactant. Due to their thermodynamic stability these systems have good shelflife, large surface area, low viscosity, and ultralow surface tension. Due to the existence of polar, nonpolar, and interfacial microdomains, these systems operate as excellent solvents of drugs; including drugs those are relatively insoluble in both aqueous and organic solvents.

Microemulsions composed of biocompatible excipients are qualified to be prospective drug delivery systems. These systems have been found to improve the bioavailability of certain drugs, e.g. orally administered labile peptides and proteins. The development of novel microemulsions to be used as biocompatible nanomaterial for biomedical and pharmaceutical applications, to improve the quality of the already existing medical devices is a very important issue. In this research project we prepared and characterized biocompatible microemulsion systems using safe ingredients.

The microemulsion systems prepared using nonionic surfactants; sucrose monolaurate (L1695) and ethoxylated mono diglyceride (EMDG) surfactants which are varying in their structures and hydrophilicity. The oils used are cyclic oils such as peppermint oil and limonene oil, linear oil such as isopropyl myristate and triglyceride oils such as caprylic capric triglyceride, olive oil, sesame oil and sunflower oil. Some of these microemulsion systems including a co-surfactants; the co-surfactants used are food, cosmetic and pharmaceutical grade short chain alcohol (ethanol) and its substitute 1, 2-propandiol (propylene glycol, (PG)) which is one of the hydrophilic simple polyols that are soluble in water but practically insoluble in the oil phase. In some of microemulsion systems, the aqueous phase contains glucose as additive.

In this research we studied the effect of adding ethoxylated mono-diglyceride surfactant (EMDG) in the phase behavior of the system W/ L1695/ Oil at different temperatures 25°C, 37°C and 45°C to see how the mixed surfactants affect on the one phase region (A_T), another objective of this research is to elucidate the microstructure of the one-phase region which was formed by electrical conductivity. A third objective was the solubilization of pharmaceutical active ingredients (Azithromycin and Cephalexin) in the formed microemulsion systems, and then study the relationship between the structural features of biocompatible microemulsions and drug solubilization using electrical conductivity.

It was found that these mixed surfactants are able to solubilize infinite amount of water in oil or mixed oils and in most of the studied systems U-type microemulsions were formulated. Electrical conductivity study enables the distinction between water-in-oil microemulsions formed at low water contents, oil-in-water microemulsions formed at high water contents and bicontinuous microemulsions formed in between the low and high water contents. The formulated microemulsions were able to solubilize large amounts of drugs (azithromycin; antibiotics and cephalixin; anti inflammatory). The drugs solubilization capacity in the microemulsions is dependent on the microstructure of the microemulsions.

ملخص البحث:

تهدف هذه الدراسة إلى تكوين مستحلبات دقيقة (Microemulsions) من مواد تدخل في الصناعات الغذائية و الدوائية ودراسة تأثير درجة الحرارة عليها ثم تشخيصها بطريقة التوصيل الكهربائي للتعرف على تركيبها الداخلي ومن ثم إذابة أدوية فيها كنوع من التطبيقات العملية لهذه الأنظمة. هذه المستحلبات الدقيقة تتكون من مواد لا تذوب ببعض (زيت وماء) لكن وجود مواد أخرى وهي المستحلبات (خافض التوتر سطحي) تجعل الزيت والماء بالعين المجردة كأنهم حالة واحدة (مادة واحدة) لكن داخليا فإن هذه الأنظمة تحتوي على جزيئات صغيرة جدا ذات حجم أقل من 100 نانوميتر. المستحلبات الدقيقة تختلف عن المستحلبات العادية كالمايونيز مثلا في أنها ثابتة حراريا وبالتالي فإنها صالحة لمدة طويلة من الزمن، شفافة، قليلة اللزوجة، التوتر السطحي لها صغير جدا وهي تعتبر مذيب جيد للأدوية خاصة تلك قليلة الذوبان بالأوساط المائية والمذيبات العضوية.

الأنظمة التي تناولتها هذه الدراسة تتكون من: ماء/ خافض التوتر سطحي لأيونية/ مساعدات خافض التوتر السطحي/ زيت. خافض التوتر السطحي المستخدم في هذه الدراسة هي سكروز استر (سكروز أحادي اللوريت L1695) و اثوكسيلاند احادي وثنائي الجليسيريد (EMDG). تعتبر هذه المواد خافض توتر سطحي لأيونية و تختلف فيما بينها بتركيبها البنائي ومدى قابليتها للذوبان في الماء، أما مساعدات خافض التوتر السطحي المستخدمة في هذه الدراسة والتي أيضا تدخل في مجالات الغذاء ومواد التجميل والصناعات الدوائية فهي مادة البروبيلين جلايكول (PG) التي تتميز بسهولة ذوبانها في الماء، لكنها عمليا غير قابلة للذوبان في الزيت. الزيوت المستخدمة و التي تناولتها هذه الدراسة هي: أولا زيوت عطرية حلقة مثل زيت النعنع (peppermint) و زيت قشور الحمضيات (R(+)-limonene)، ثانيا زيوت خطية التركيب مثل الأيزوبروبيل مريستيت (isopropyl myristate)، ثالثا زيوت ثلاثية الجليسيريد مثل زيت الفستق (caprylic capric triglyceride)، زيت الزيتون، زيت السمسم ، زيت الصويا وأخيرا زيت عباد الشمس.

من خلال هذه الدراسة وجد أن المستحلبات الدقيقة المبنية في تركيبها على زيت النعناع تعطي اكبر مساحة وحيدة الحالة في رسومات الحالات للمستحلب الدقيق من أي زيت آخر، كذلك تم دراسة اثر استخدام خليط من المركبات المستحلبة مثل : ايسترات السكروز و جليسيريد أحادي و ثنائي اوكسيد الايثيلين بنسب مختلفة عوضا عن مركب منفرد في تكوين المستحلبات الدقيقة حيث وجد أن استخدام خليط من هذه المركبات يعطي مساحة وحيدة الحالة (مستحلبات دقيقة) على منحني الرسم اكبر بالمقارنة مع استخدام مركب منفرد، كما وجد أن خليط من خوافض التوتر السطحي (EMDG + L1695) على نسبة 1:1 أعطى أحسن مساحة للمستحلبات الدقيقة على درجات الحرارة المدروسة. وذلك عن طريق حساب المساحة الكلية للمنطقة المكونة من طبقة واحدة (A_T).

ومن الأهداف الأخرى لهذه الدراسة هو تبيان و توضيح البناء الجزيئي الدقيق للمنطقة المكونة من طبقة واحدة، وذلك باستخدام جهاز التوصيل الكهربائي، حيث وجد أن التوصيل الكهربائي للمستحلبات الدقيقة سواء كانت مبنية من مركب مستحلب منفرد أو مزيج من المستحلبات تعتمد بشكل أساسي على محتوى الماء داخل المستحلبات الدقيقة. كذلك وجد أن أعلى قيمة للتوصيل الكهربائي للمستحلبات الدقيقة التي تم دراستها تحدث عندما تزيد كمية الماء على الزيت مما يدل على تحول في شكل التركيب الدقيق للمستحلبات الدقيقة من (ماء في زيت) إلى (زيت في ماء)، كما لوحظ أيضا بالنسبة لجميع الأنظمة التي تم دراستها أن قيم التوصيل الكهربائي تزيد بزيادة درجة الحرارة و ساعد أيضا قياس التوصيل الكهربائي بالتميز ما بين مستحلبات دقيقة (ماء بزيوت) من مستحلبات دقيقة (زيت بماء) من مستحلبات دقيقة متصلة التركيب.

أما الهدف الثالث لهذه الدراسة هو استكشاف مدى قدرة المستحلبات الدقيقة على إذابة عقاقير (ذات ذائبية منخفضة جدا في الماء) مثل السيفالكسين والأزيتروميسين في تركيبها الداخلي والمبنية في تركيبها على مزيج من المركبات المستحلبة (السكروز أحادي اللوريت و ايثوكسيلات أحادي وثنائي الجليسيريد) بوجود أو بعدم وجود البروبيلين جليكول كمذيب مساعد، حيث وجد أن سعة الذائبية للعقاقير المستخدمة في الدراسة تزيد مع زيادة محتوى الماء في المستحلب الدقيق إلى حد معين ثم تنخفض وهذا دليل على حدوث تغير في التركيب الداخلي للمستحلبات الدقيقة مما يؤثر على مستوى ذائبية الأدوية.

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1. Introduction:

Microemulsions (ME) are spontaneously forming, thermodynamically stable, homogeneous low viscous, and optically isotropic solutions. These macroscopic homogeneous mixtures are heterogeneous on a nanometer scale (Kumar and Mital, 1999). Schulman's group was the first who scientifically described microemulsion in 1943 (Hoar and Schulman, 1943) though the concept was there in the patent literature in mid-1930s. The term "microemulsion" was first coined in 1959 by Schulman and his group (Schulman, Stockenius and Prince, 1959). Prior to that, different, terms like transparent emulsion, swollen micelle, micellar solution, and solubilized oil were used for such systems.

ME systems consist from oil, water, and an amphiphile (surfactant molecules) that brings down the water/oil interfacial tension (IFT) to a very low value. Originally, it was thought that there exists a negative IFT which imparts stability to microemulsion (Prince, 1977). It is accepted that the IFT between oil and water is reduced to a very low value by the presence of an amphiphile, but there are many instances though, where the amphiphiles do not bring the IFT down to the required very low value and another substances need to be added to obtain the required IFT for the formation of a stable microemulsion such as short chain alcohols. This means that in most cases the microemulsions are four component systems, these are water, oil, surfactant, and a short chain substance called a cosurfactant.

The basic difference between emulsion and microemulsion is the fact that in the former the droplet size is in the region of micrometer whereas in the latter it is much smaller (<100 nm). There can be some systems where the droplet sizes lie in between, and such systems are called miniemulsions. Both miniemulsions and emulsions are not thermodynamically stable but they are kinetically stable. This means that the stability of a microemulsion formed under a given condition of temperature and pressure is time independent whereas the stability of a formed emulsion or miniemulsion under a given condition is a function of time. Further, for the formation of emulsion, the requirement of surfactant is low (about 1%–2%) whereas for microemulsion the surfactant required is high. The formation of microemulsion is a spontaneous process, requiring no energy or a very small amount of energy, whereas for emulsion and miniemulsion the energy requirement is appreciable (Spernath, Yaghmur, Aserin, et al, 2002). Table 1 illustrates these differences.

Table 1.1: Comparison of Coarse Emulsions and MEs (Tenjarla, 1999).

Property	Microemulsion (ME)	Coarse Emulsion
Disperse phase droplet size	Less than 100 nm	0.2–10 μm
Visual appearance	Transparent to translucent	Turbid to milky
Stability	Thermodynamically stable	Thermodynamically unstable
Formation	Spontaneous	Requires energy input
Microstructure	Droplets or bicontinuous	Droplets

ME systems can be one of three types depending on the relative ratios of the constituting components: oil-in-water (o/w ME) systems comprise water as the continuous medium; water-in-oil (w/o ME), where oil is the continuous medium and bicontinuous microemulsion (Garti, Fanun, Aserin, et al, 2001). The simplest representation of the ME microstructure is with reference to the droplet model in which an interfacial film comprising an amphiphile (surfactant/cosurfactant) molecules surrounds the dispersed droplets (Figure 1).

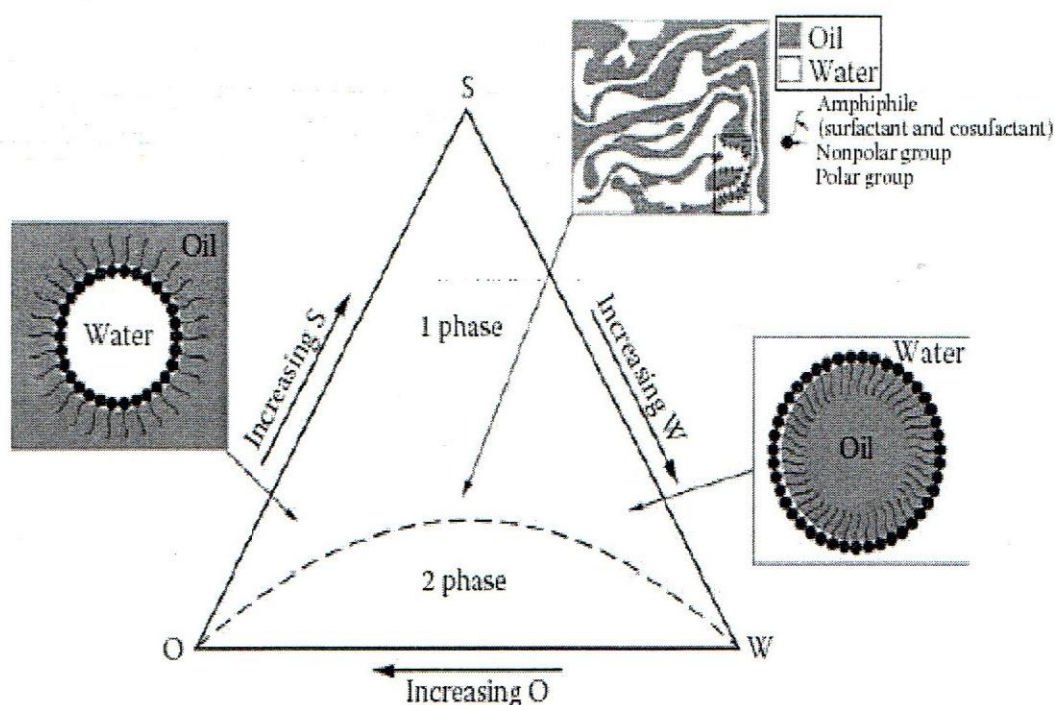


Figure 1.1: Theoretical ternary phase diagram outlining the region of existence of one phase and two-phase systems. Note the illustrative representation of the droplet w/o, droplet o/w, and bicontinuous MEs. O, oil component; W, water component; S, amphiphile component (surfactant/cosurfactant). (Fanun; 2009)

Microemulsions, in general, exist in equilibrium with either excess oil or excess water or both. Winsor (Winsor, 1948) has classified these different types of systems. When oil-in-water (o/w) microemulsion is in equilibrium with excess oil, it is known as Winsor **I**. The w/o microemulsion in equilibrium with excess water is called Winsor **II**. A microemulsion maintaining equilibria with both oil and water is called Winsor **III**. This is also termed as bicontinuous middle phase microemulsion in which both w/o and o/w dispersions remain simultaneously present. It is possible to get a microemulsion system which is not in equilibrium with either oil or water. This is known as Winsor **IV** system. All these four systems are illustrated in Figure 2.

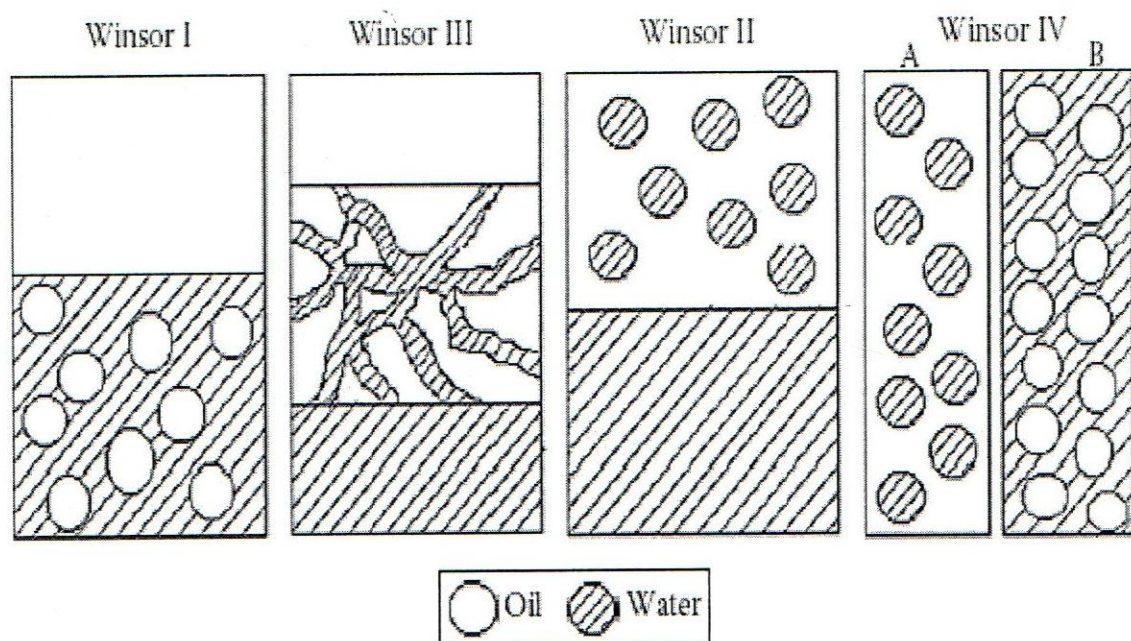


Figure 1.2: Different forms of Winsor structures (Winsor, 1948).

Due to their unique properties which include thermodynamic stability, high solubilization power, low interfacial tensions, transparency, and low viscosity, microemulsions have been used in a variety of technological applications, including environmental protection, nanoparticle formation, personal care product formulations, drug delivery systems, and chemical reaction media (Solans and Kunieda, 1997). In most industrial applications, the use of mixed surfactants system is preferred over the use of pure surfactant-based systems (Ogino and Abe, 1992). The determinations of the phase behavior of water/mixed surfactants/oil system quantitatively is of eminent significance for further investigation and possible applications. The study of the phase behavior of mixed nonionic surfactants in water and oil demonstrated that the amount of surfactants present at the water–oil interface determines the extent of water and oil mutual solubilization (Fanun, 2008).

This amount of surfactants depends on factors like surfactant's chemical structure and hydrophilicity, the monomeric solubility of surfactants in oil and water, and the presence of additives. It was found that the water-in-oil (w/o) solubilization increases dramatically when nonionic surfactants, whose hydrophilic–lipophilic balances are far separated, are mixed (Kunieda, Nakano and Akimura, 1995). At constant temperature, the hydrophilic–lipophilic balances of mixed surfactants in surfactants monolayers inside the microemulsion are the same in the case where oil is fixed. It was also found that the total surfactant concentration needed to solubilize an equal amount of water and oil increases with the increase in the lipophilicity (molecular weight) of the oil (Kunieda, Nakano and Pes, 1995).

2. Conclusions

This research work investigated the following subjects in the domain of mixed surfactants systems: (1) Study of the phase behavior and exploring the factors affecting it. (2) Elucidation of the microstructure of the mixed surfactants systems. (3) Revelation of the possibility of solubilization of pharmaceutical active ingredients in the microemulsions formed using these mixed surfactants. In the following we redraw the general conclusions varied from these subjects; the water solubilization capacity study revealed that mixed surfactants improve the water solubilization capacity in the microemulsions compared to the microemulsion systems based on single surfactants. The molar ratios of mixed surfactants play an important role in determining the maximum water solubilization; the use of mixed surfactants (L1695+EMDG) at weight ratio equals unity (1:1) increases the water maximum solubilization indicating a synergistic effect. The synergism phenomenon may be a result of better interface organization (orientation) of the mixed surfactants around the oil droplets that allows better interfacial solubilization (enhanced partitioning of the surfactant at the interface). The chemical structure of the oil affects its penetration in the surfactants palisade layer and determines the extent of water solubilization. Results are reported on the formulated biocompatible microemulsions based on mixed surfactants and the mixed oils; it was found that using the weight ratio of mixed oils equal (1/1) improved the water solubilization capacity in the microemulsions. Also it was found that introducing glucose as additives to microemulsion formulations instead of propylene glycol don't affect the area of one phase region but when introducing calcium oxide (CaO) to the microemulsion formulations the area of one phase region decreases. The increase in temperature from 25°C to 45°C affect on microemulsion formation and then the area of one phase region.

Different microemulsion systems were investigated using electrical conductivity; the electrical conductivity increases with the increase of water content and with increase of temperature. Generally, three different regions were identified by this technique the first region represents water-in-oil microemulsion (w/o), the second region represents bicontinuous structure while the third region represents oil-in-water microemulsion (o/w). These results also revealed the presence of a percolation threshold which indicates a structural transition from water-in-oil to percolated structure previous to the formation of bicontinuous microstructure. When the microemulsion systems based on mixed surfactants, then the electrical conductivity values higher than systems based on single surfactant and at mixing ratio of mixed surfactants equals unity, the electrical conductivity is the highest than other ratio. In some of microemulsion systems investigated electrical conductivity results revealed the formation of rigid microemulsion in which the electrical conductivity increases initially with aqueous phase content increase then decreases then again increases, these rigid microemulsions are very important systems in industrial purposes. For drug loaded microemulsion systems; the electrical conductivity results showed that the electrical conductivity of loaded systems is higher than free systems.

The solubilization capacity of azithromycin and cephalexin in microemulsion systems was higher than any single component that formed the microemulsion. It was found that the solubilization curves for azithromycin and cephalexin showed different regions, region at low water content (0wt% to 30wt.%) which is a w/o microemulsion, in this region the solubilization capacity was the highest due to high surface area and high oil content. Region which contains bicontinuous microemulsion and this region extent from 30wt% to 70wt% water content, in this region the solubilization capacity almost remains constant due to the fact that surface area remains constant at this stage and the region which contains o/w microemulsion and this region extent from 70wt% to 100wt% water content, in this region the solubilization capacity was sharply decreased due to that small amount of oil exist in the core of droplets and the interface became convex toward the oil resulting in very low solubility in the core and poor accommodation of the drugs at the hydrophilic interface. The solubilization capacity of azithromycin is higher than cephalexin for the same microemulsion system and this due to its chemical structure.