Microemulsion System Based on Nonionic Surfactant Igepal CO-520 for Encapsulation of Vitamin E

Dharmesh Varade

School of Science, Navrachana University, Vasna Bhayli Road, Vadodara 391410, Gujarat, India.

Received: 1 July 2023 Revised: 13 December 2023 Accepted: 27 December 2023 Published: 28 December 2023

*Corresponding Author: dharmesh.varade@nuv.ac.in

https://doi.org/10.5281/zenodo.11438621

Abstract

The viability of formulating a novel reverse microemulsion with a biocompatible nonionic surfactant and nontoxic oils has been revealed. Oil soluble nonionic pentaoxyethylene (5) nonylphenyl ether (Igepal CO-520) as surfactant and caprylic acid (CA), oleic acid (OA), isoamyl acetate (IAA) and isopropyl myristate (IPM) are used as oil phase. Ethanol, propanol and diethylene glycol monoethyl ether (carbitol) are incorporated as co-surfactants. Igepal CO-520 is solubilized in oil phase and ternary/pseudoternary phase diagrams are created by titration with water and the effect of various co-surfactants, oils, and the mixing ratio of surfactant/co-surfactant (S_{mix} = 1:2, 1:1 and 2:1) on the microemulsion region is systematically investigated. In the absence of co-surfactants, Igepal CO-520 forms clear and stable microemulsion with all the oils, but greater microemulsion area is perceived only with IPM and the samples are slightly viscous. This microemulsion region of IPM is further increased in the presence of carbitol co-surfactant and the samples are clear, stable, and non-viscous. The electrical conductivity and viscosity measurements are made to examine the microstructural changes within the microemulsion region. Three dissimilar phases such as oil in water (o/w), bicontinuous (BC) and water in oil (w/o) microemulsion are observed. Dynamic light scattering (DLS) is used to study the droplet size of microemulsion samples. We primarily focused on microemulsion formation and understanding its kinetic and thermal stability. Furthermore, the microemulsion depicts remarkable encapsulation ability for vitamin E (α-tocopherol). The results obtained in this study have great potential for applications of this microemulsion in personal care, food and pharmaceutical products.

Keywords

Igepal CO-520, Microemulsion, Triglyceride oils, Phase diagram, Vitamin E, Carbitol

Introduction

Microemulsions are isotropic liquid of oil and water frequently stabilized by surfactants and co-surfactants. They have attracted a great deal of attention due to their exclusive properties like ultra-low interfacial tension, nano-sized droplets (<100 nm), transparency, extraordinary stability, and ease of formation[1-4]. Due to these, microemulsions have been used to encapsulate and deliver water-insoluble active ingredients in the pharmaceutical[5-7], food[8-10], and agrochemical industries[11-13]. Also, they have also been utilized as colloidal delivery systems for biological active ingredients like fat-soluble vitamins, nutraceuticals and drugs[14,15]. While there are numerous uses of microemulsion, there are notable challenges related to their applications. The crucial challenge is the usage of some conventional artificial surfactants that can cause adverse effects when used for the formulation of microemulsions. Many of the effective surfactants and/or cosurfactants do not have a pharmaceutically acceptable toxicity profile. Also, high concentration of surfactant is requisite for the formation of microemulsion which makes it expensive. Moreover, occurrence of toxic, flammable and other volatile organic solvents in the oil phase of microemulsion imposes the safety measures. Hence, it is the requirement of green chemistry and engineering to formulate microemulsion systems by choosing appropriate biocompatible surfactants, and renewable nontoxic oils. Igepal is polyoxyethylene based nonionic surfactant series and is extensively used in technical applications either as single surfactant or mixed surfactant systems with anionic and cationic surfactants[16,17]. Igepal CO-520 is pentaoxyethylene (5) nonylphenyl ether with oxyethylene chain as hydrophilic and branched alkyl chain as hydrophobic moieties in its structure having HLB 10. Igepal is a specially tailored nonylphenyl ether compound with significant advantages, making it the optimal choice for a broad spectrum of applications like cleansing, emulsifying, and processing. It has been reported that with increasing number of exthoxylated groups, the biodegradability of surfactant also increases[18]. Therefore, utilizing this surfactant over other conventional nonionic surfactants for preparing microemulsions using biocompatible oils and co-surfactants can be immensely useful. Moreover, the HLB 10 of Igepal CO-520 leads to the formation of reverse (w/o) microemulsions. The water molecules solubilized in the core of the reverse microemulsion systems have properties different from bulk water. Also, the water in the core of nonionic reverse microemulsions is not

perturbed by charges due to the presence of ethylene oxide chain as hydrophilic moiety. This makes reverse microemulsions potentially useful in industry and biologically important systems. The preliminary studies with Igepal CO-520 reported the water solubilization capacity in the reverse micelles and the factors influencing the solubilization[17-19]. One of the studies also reported the synthesis of lanthanide fluoride nanoparticles using Igepal based microemulsion[20]. Likewise, silica coated magnetite nanoparticles synthesis using reverse microemulsion method with Igepal CO-520 has been reported[21]. However, no study has been reported on the formulation of green microemulsion using Igepal CO-520 for wide applications in pharma or personal care to the best of our knowledge.

In this context, we have made an attempt to formulate microemulsions using Igepal CO-520 as surfactant and trigylcerides such as caprylic acid (CA), oleic acid (OA) and carboxylic acid esters like isoamyl acetate (IAA) and isopropyl myristate (IPM) as biocompatible oil phase. However, the major challenge in using these oils, especially long chain triglycerides is the formation of undesirable phases such as gel or liquid crystalline phases during microemulsion formulation[22]. Hence, to overcome this, we used carbitol and short chain alcohols like ethanol and propanol as co-surfactants for creating microemulsion with Igepal CO-520. We primarily focused on microemulsion formation and understanding its kinetic and thermal stability. The phase diagrams are determined to recognize microemulsion region and appropriate composition. The variations in the microstructures of microemulsion are considered using electrical conductivity and viscosity measurements. We examined the influence of oils and co-surfactants on the properties of microemulsions. We have also explored the encapsulation of vitamin E (α-tocopherol) in the Igepal CO-520 microemulsion for greater bioavailability of vitamin E. This study might have repercussions in the designing of reverse microemulsions with Igepal CO-520 surfactant expanding the prospect of their applications.

Experimental

2.1 Materials

Pentaoxyethylene (5) nonylphenyl ether (Igepal CO-520) and vitamin E (> 96%) is purchased from Sigma-Aldrich. Isoamyl acetate (> 99%) is from TCI Pvt. Ltd. (Tokyo, Japan). Propanol (> 99%), carbitol (97%), isopropyl myristate (95%) and oleic acid (65%) are from HPLC Pvt. Ltd. (Mumbai, India). Caprylic acid (> 99%) is obtained from SRL (Mumbai). Ethanol (> 99%) is acquired from HiMedia Laboratories Pvt.

Ltd. (Mumbai). The structures of surfactants and oils are shown in **Table 1**. All the solutions are prepared in deionized water and kept for 24 h before any measurements.

2.2 Methods

Phase Behavior: Phase behavior studies for microemulsion are performed by constructing ternary and pseudoternary phase diagram by the method that is previously reported[1]. Microemulsion systems were prepared by mixing oil with the mixture of surfactant and cosurfactant, and water was added precisely into oily phases with magnetic stirring (300 r/min) at 27 °C. Briefly, the surfactant or mixture of surfactant/co-surfactant (S_{mix} = 1:2, 1:1, and 2:1) are mixed with oil in different proportions keeping the total weight constant for ternary and pseudoternary diagrams respectively at 27 °C. These mixtures are then titrated with drop-wise addition of water till the solution turns turbid and composition of each of the components is marked as end point for determination of microemulsion area. In this study, triglyceride oils such as caprylic acid, oleic acid and carboxylic acid esters like isoamyl acetate and isopropyl myristate are used as oil phase. Nonionic surfactant Igepal CO-520 is used as surfactant and ethanol, propanol, and carbitol are used as co-surfactants. All the samples are kept for 24 h at 27 °C to attain equilibrium.

Conductometry and Viscometry: The electrical conductivity (EC) of microemulsions is measured by a conductivity meter from Horiba Japan. Viscosity measurements are performed with Vibro-viscometer (SV-10 Series, A&D Company, Japan) using the tuning-fork vibration method. The samples are retained at 27 ± 1 ^{o}C for 24 h prior to measurements.

Dynamic Light Scattering: Micellar sizes are determined into the quartz cell by Zetasizer Nano-ZS, ZEN3600 from Malvern instruments Ltd at 27 ± 1 ^{o}C.

UV-visible Spectroscopy: UV-visible absorption spectra are recorded with samples in a quartz cuvette (1 mm), using a Hitachi U-4100, UV-vis double-beam spectrometer. The absorption spectrum is obtained in the wavelength range of from 200–500 nm. Pure microemulsion sample without vitamin E is used as reference in the UV spectroscopy analysis.

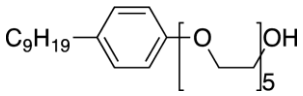
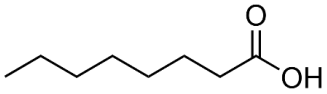
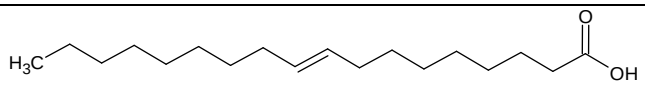
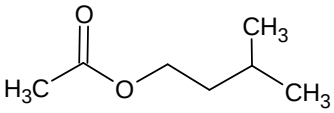
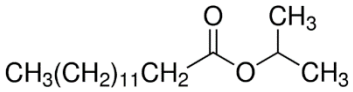
Name of Surfactant/Oils	**Structure**
Pentaoxyethylene (5) Nonylphenyl Ether (Igepal CO-520)	
Caprylic Acid (CA)	
Oleic Acid (OA)	
Isoamyl Acetate (IAA)	
Isopropyl Myristate (IPM)	

Table 1: Chemical Structure of Surfactant and Oils.

Results and Discussion

Phase Behavior

Figure 1 depicts the ternary phase diagram of water, nonionic surfactant Igepal CO-520 with triglyceride oils like caprylic acid (CA), oleic acid (OA) and carboxylic acid esters like isoamyl acetate (IAA) and isopropyl myristate (IPM) determining the microemulsion (μE) area as a function of composition at 27 ± 1 ^{o}C. The mixture of Igepal CO-520 and various oils in different proportions is taken in glass vials at fixed amount and

then titrated with water by vigorous stirring till the solution turned turbid. The samples are then observed in cross polarized light for determination of monophasic/biphasic system after 24 h. If the samples turned translucent or turbid followed by phase seperation, these are assumed biphasic. The clear, transparent samples are identified as microemulsion and composition of these solutions are marked as points in phase diagram. The total area concealed by these points in ternary phase diagrams is considered as microemulsion (μE) region and is shown as shaded area in Figure 1.

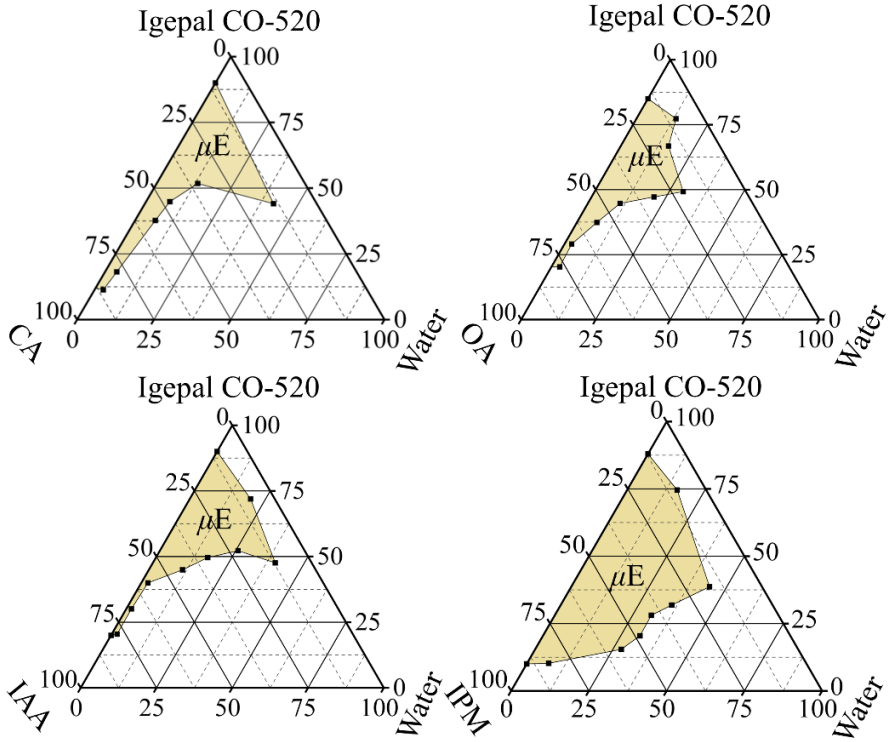


Figure 1: Ternary phase diagrams of water, Igepal CO-520 with various oils like caprylic acid (CA), Oleic acid (OA), Isoamylacetate (IAA), and Isopropyl myristate (IPM) at 27 ± 1 ºC. Microemulsion (μE) region is shown by shaded area.

Effect of different oils: The effect of different triglyceride oils (CA, and OA) and carboxylic acid ester oils (IAA and IPM) on microemulsion area is studied. The microemulsion area obtained for Igepal CO-520 is very narrow with triglyceride oils and slightly broader for carboxylic acid esters. This could be due to the fact that triglyceride oils, especially long chain OA can form undesirable phases like macroemulsions, liquid crystalline phase or spongy phases which lead to decrease in microemulsion area[22]. Carboxylic acid esters

IPM being more lipophilic than IAA could form greater microemulsion region with Igepal CO-520 having HLB 10. It is a well-known fact that low HLB value of surfactant leads to formation of water-in-oil (w/o) microemulsion region near the oil/surfactant boundary in the ternary phase diagram[23] as depicted in Figure 1. Igepal CO-520 at concentration of 35 wt% in IPM could solubilize 45 wt% of water in the core of the reverse micelles and some amount might be present as bulk water leading to the formation of wider microemulsion area. This could be due to greater polarity of the IPM which led to solubilization of greater amount of water in the core of the reverse micelles[24-25]. Hence, we have selected IPM as an oil phase for further studies. Also, the samples in the microemulsion region with IPM are slightly viscous. This could be due to the fact that with increasing water content in the microemulsion system, the number density of the reverse micelles increases leading to stronger interactions among the reverse micelles making the samples slightly viscous.

Effect of co-surfactants: In this study, we used several different co-surfactants with Igepal CO-520 surfactant to understand the effect of co-surfactants on the microemulsion area. **Figure 2** depicts the aqueous pseudoternary phase diagrams of various co-surfactants ethanol, propanol and carbitol with Igepal CO-520 at fixed weight ratios of surfactant/co-surfactant (S_{mix} = 1:1) and IPM as oil. Phase diagrams are prepared using the same methodology as mentioned above by taking pre-weighed amounts of S_{mix} and oil in different ratios at fixed weight and titrating with pure water. Visual inspection is done using cross polarized light to determine monophasic/biphasic region. The shaded area in Figure 2 represents the microemulsion (μE) region in pseudoternary phase diagram.

It is found that microemulsion area increased towards the water-rich region in the presence of carbitol as compared to ethanol and propanol. This is ascribed to the structure of carbitol which can position in between the surfactant tails of the reverse micelles forming mixed adsorption film on the interface and thereby increasing the flexibility and fluidity of the interfacial film formed by Igepal CO-520 and carbitol. This resulted in an upsurge of miscibility of an aqueous and organic phase with each other. Thus, greater microemulsion region is formed with IPM as oil phase and S_{mix} = 1:1 (Igepal CO-520: carbitol). However, microemulsion area of IPM and Igepal CO-520 decrease in the presence of co-surfactants like ethanol and propanol. This could be due to the fact that short chain alcohols like ethanol and propanol being more hydrophilic are solubilized in the core of the reverse micelles along with water rather than being inserted on the interfacial layer leading to reduction in microemulsion area[26]. Above results indicate that only IPM could

form greater microemulsion area. This could be attributed to the compatibility of the IPM with the given nonionic Igepal CO-520 as surfactant and carbitol as co-surfactant.

Additionally, it is observed that in the occurrence of co-surfactant, viscosity of the microemulsion samples reduces significantly. The inclusion of co-surfactant causes the reduction in the bending modulus of the surfactant film leading to an increase in the elasticity of the surfactant film to curve more readily towards oil or water phase. This leads to decrease in viscosity of the microemulsion sample by preventing the formation of more rigid structures like liquid crystalline or gel like phases[27,28]. From these phase behavior studies, we selected the combination of Igepal CO-520/carbitol (S_{mix}) and IPM as oil phase which formed the greatest microemulsion area for further characterization of microemulsion.

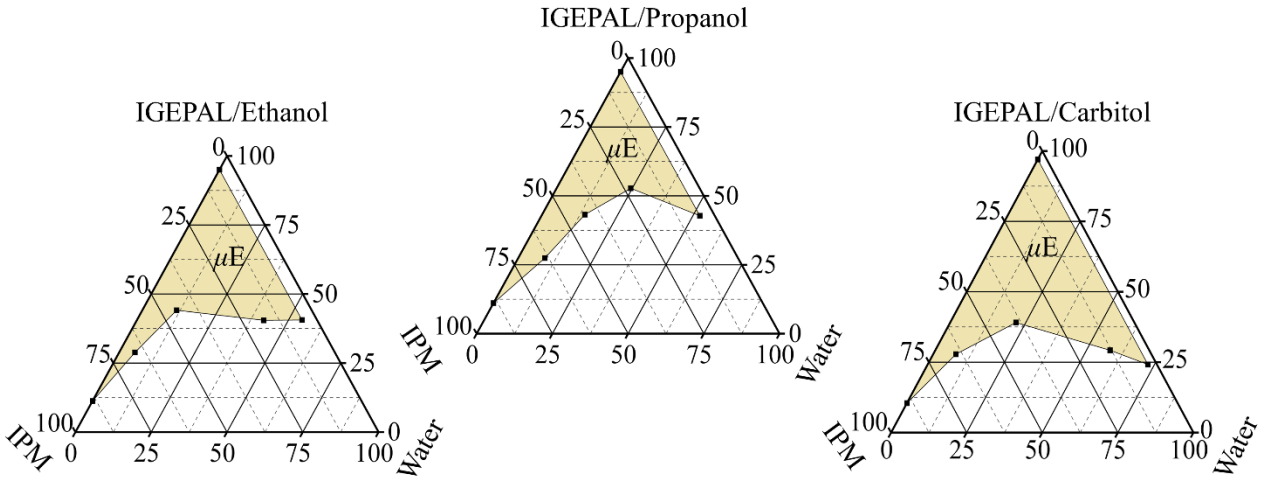


Figure 2: Pseudoternary phase diagrams of Igepal CO-520 with different co-surfactants ethanol, propanol, and carbitol at fixed surfactant/co-surfactant weight ratios (S_{mix} = 1:1), water and isopropyl myristate (IPM) as oil. Microemulsion (μE) region is shown by shaded area.

Effect of surfactant/co-surfactant (S_{mix}) ratio: To explore the effect of surfactant/co-surfactant ratio (S_{mix}) on the microemulsion area, we studied the pseudoternary phase behavior of Igepal CO-520/carbitol in different mixing ratios of 2:1, 1:1 and 1:2 with IPM as oil phase as represented in Figure 3. It is observed that with Igepal CO-520/carbitol in 2:1 ratio, the microemulsion region is very narrow and only limited amount of water (62 wt%) could be solubilized in the microemulsion system. This could be due to higher content of surfactant leading to formation of smaller microemulsion droplets but would also incorporate more amount of oil reducing the solubilization of water in the oil phase causing an imbalance at the interface,

consequently resulting in the decreased microemulsion area. The phase diagram depicted an almost similar microemulsion region for S_{mix} 1:2. Co-surfactant can exist on the interface layer when added in adequate amounts, but higher content of co-surfactant can disrupt the interfacial film between the oil/water interfaces leading to demulsification of microemulsions[29]. Surfactant/co-surfactant ratio of S_{mix} = 1:1 is showing optimum ratio with extended microemulsion area and is selected for the complete characterization of the microemulsion system.

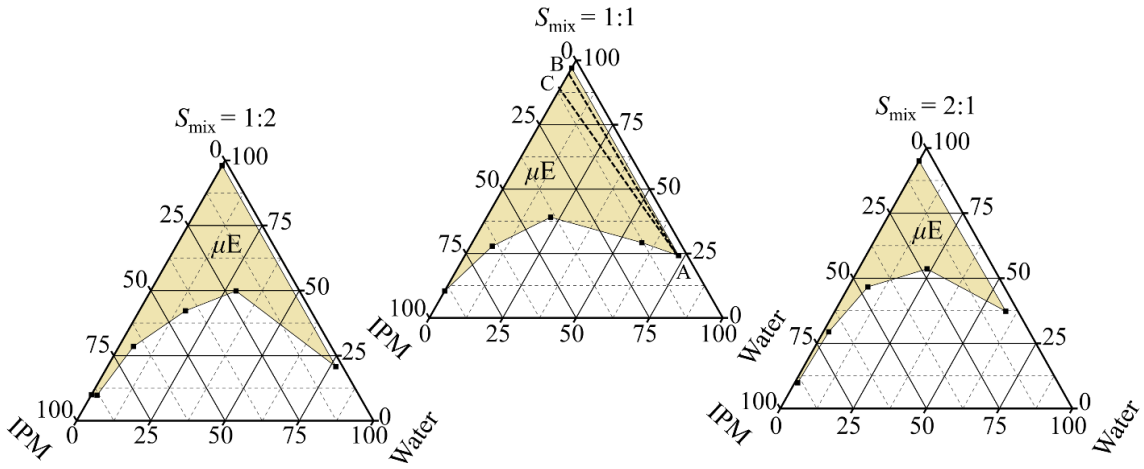


Figure 3: Pseudoternary phase diagrams of Igepal CO-520/carbitol with IPM at S_{mix} 2:1, 1:1 and 1:2 respectively at 27 ºC. Microemulsion (μE) region is shown by shaded area.

All the microemulsion samples obtained from the phase behavior studies are crystal clear, stable and depicting low viscosity. These samples are prepared by simply mixing the components at a specific composition without any high energy input. The microemulsions formulated from nonionic Igepal CO-520 are biocompatible, formed spontaneously, posess excellent thermodynamic stability and easy to scale-up. To elucidate the structural variations occurring in the microstructures of the microemulsions upon dilution with water, characterization techniques like conductivity and viscosity of the samples are performed.

Microstructural Transition

Electrical conductivity and viscosity are structure-sensitive properties[26,30]. Hence, the electrical conductivity (EC) and viscosity studies are performed along the dilution line 'AB' and 'AC' representing the (S_{mix}/oil) in 9:1 and 8:2 ratios respectively where Igepal CO-520/carbitol (S_{mix} = 1:1) with water content varying from 10-70 % to determine the structure of microemulsion.

Electrical conductivity (EC) measurements: The variation in EC with increasing water content along both dilution line 'AB' and 'AC' is represented in Figure 4a. EC increased gradually from 16 to 75 mS/cm from 10-30 % water content. The lower EC values for nonionic system indicated the presence of very few and discrete reverse micelles existing in continuous non-conducting oily phase and EC values almost similar to pure oil. EC amplified linearly to 175 mS/cm with moderate increase in water content from 30-50 %. This exemplifies the percolation phenomena of the aqueous phase into network like conducting channel caused by the attractive interactions among the water droplets resulting in increased EC[31]. This indicates transition of water-in-oil (w/o) microemulsion to bicontinuous (BC) microemulsion. The EC reached maximum 200 mS/cm at 70 % water content indicating the existence of oil-in-water (o/w) microemulsion droplets in continuous aqueous phase. This increase in EC values with increasing water content is a characteristic of microstructure transition from w/o microemulsion – bicontinuous microemulsion – o/w microemulsion.

Viscosity measurements: The change in viscosity with increasing water content along the dilution line 'AB' and 'AC' is represented in Figure 4b. It could be observed that at lower water content from 10-30 %, higher viscosity in the range of 59-47 mPa is obtained and there is not much variation in viscosity of the microemulsion samples indicating the presence of w/o microemulsion droplets. The higher viscosity could be due to better interaction of S_{mix} with oil phase. However, the viscosity along dilution line 'AB' is slightly higher than 'AC'. This could be due to higher surfactant content leading to formation of greater number of w/o microemulsion droplets along the dilution line 'AB' leading to higher viscosity. With further increase in water content from 30-70 %, there is a steady decrease in viscosity of the samples. This is due to the increase in size of the droplets and the interaction between the surfactant and oil phase is loaned[26]. The sharp decrease in viscosity above 50 % water content is due to formation of discrete o/w microemulsion droplets. The structural variations from w/o – bicontinuous – o/w microemulsion with increase in water content could be interpreted by combining EC and viscosity studies.

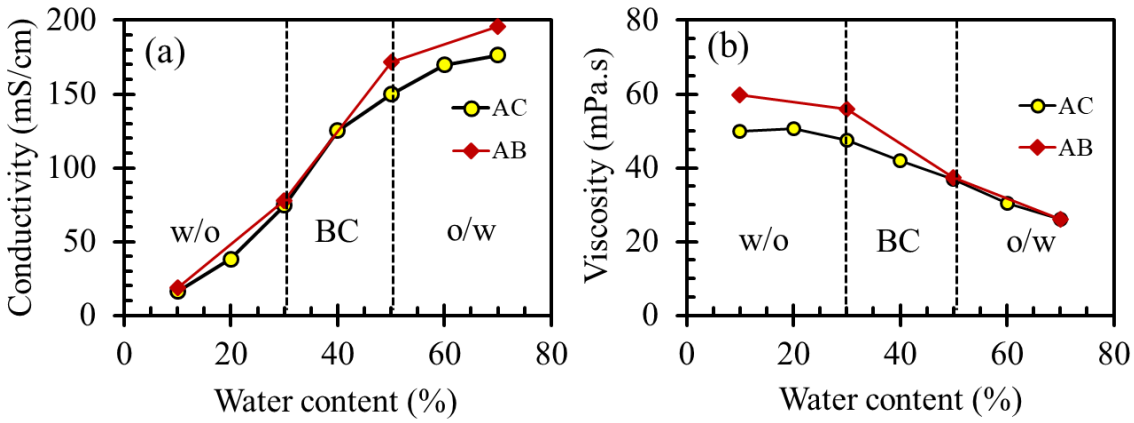


Figure 4: (a) Variation in electrical conductivity and (b) Changes in viscosity along the dilution line 'AC' (S_{mix}/oil = 8:2) and 'AB' (S_{mix}/oil = 9:1) as displayed in Figure 3.

Effect of dilution and S_{mix}/oil ratio: Figure 5a depicts dynamic light scattering (DLS) measurements along the dilution line 'AC' as marked in Figure 3. This dilution line is selected due to lower viscosity and incorporation of lesser surfactant and greater oil. The droplet sizes of all the microemulsion samples increased from 5 nm to 32.7 nm. The increase in the droplet size could be due to the incorporation of greater amount of water in the core of the reverse micelles with increasing water content along the dilution line. Also, it could be observed that there is trivial variation in the droplet size from 30-50 % water content due to existence of clustering of reverse micelles with water in its core representing the bicontinuous structure. The small droplet sizes led to highly stable microemulsion. Moreover, the PDI values fluctuated in the range of 0.1-0.7 indicating the uniform distribution of microemulsion droplets leading to greater stability of microemulsion[32].

The effect of (Igepal CO-520/carbitol = 1:1) S_{mix}/oil (IPM) is investigated using DLS studies. Figure 5b represents S_{mix}/oil in 9:1, 8:2 and 7:3 with fixed amount of water (60 wt%) content. No specific trend is observed in the droplet sizes with varying S_{mix}/oil ratio. The smallest droplet size of 11 nm is obtained at 8:2 ratio. At 9:1 and 7:3 ratios, droplet sizes of 18 nm and 21 nm are obtained respectively. This indicates that at fixed water content, the increasing concentration of surfactant did not have effect on the droplet size of the microemulsion.

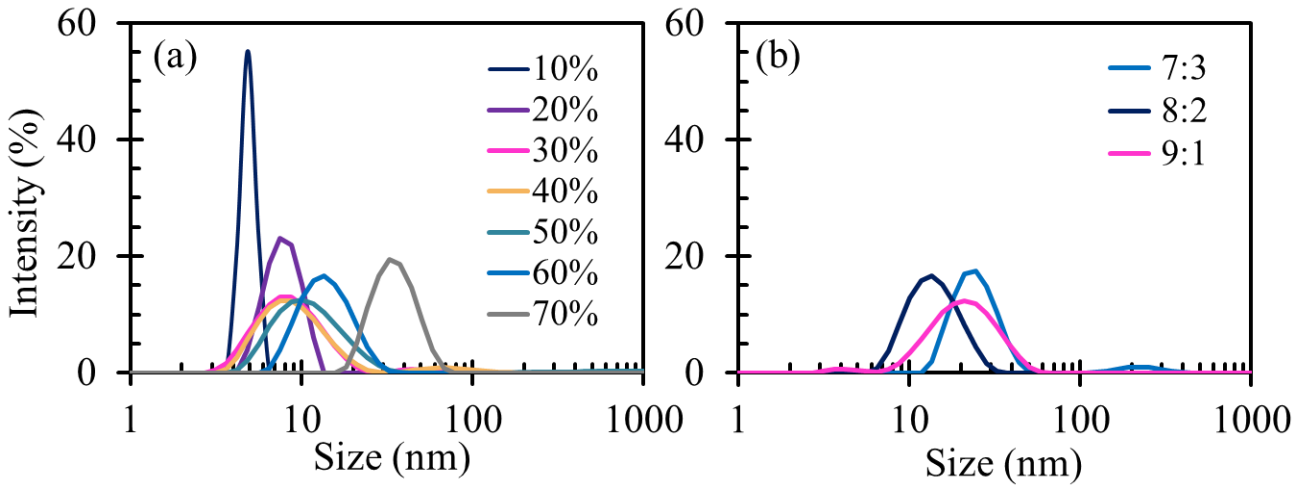


Figure 5: (a) Mean particle size of samples along the dilution line AC (shown in Figure 3) from 10–70 wt% water content, (b) mean particle size of samples at 60 wt% water content for (Igepal CO-520/carbitol = 1:1) S_{mix}/oil (IPM) in 7:3, 8:2 and 9:1 ratio.

DLS studies are also implemented to comprehend the stability of microemulsions with 70 wt% water along the dilution line 'AC' and then 500 times, 1000 times and 5000 times dilution with water. It is found that at higher dilution values, the size of the droplets increased abruptly >1000 nm for all the samples with 70 wt% and 30 wt% water. This study indicated that the microemulsion samples are not very stable at higher dilution values.

Stability measurements Stability of microemulsion plays a vital role for its several applications. Hence, we performed thermodynamic, kinetic and storage stability of microemulsions.

Thermodynamic stability: The thermodynamic stability of microemulsion samples is conducted by placing the samples at three different temperatures of 27 °C, 45 °C and 4 °C for 48 h and repeated this cycle thrice. If the samples exhibited phase separation or turned turbid, they are considered as unstable formulations. Figure 6 represents the thermodynamic stability of microemulsion samples with (Igepal CO-520/carbitol = 1:1) S_{mix}/oil (IPM) at 8:2 ratios with 10-70 wt% water content as represented on the dilution line "AC". It is clearly seen from the images that all the samples are extremely stable at 27 °C and at 4 °C. However, at higher temperature (45 °C), microemulsion sample containing 70 wt% water content exhibited phase separation (PS) as indicated in the Figure. This concludes that all other samples are thermodynamically stable.

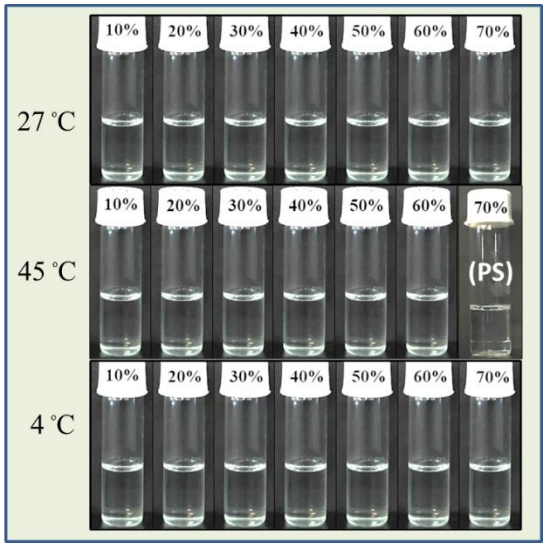


Figure 6: Thermodynamic stability of microemulsions containing (Igepal CO-520/carbitol = 1:1) S_{mix}/oil (IPM) at 8:2 with water content from 10–70 wt% at 27 °C, 45 °C and 4 °C.

Storage stability: The microemulsion samples with same composition as mentioned above for thermodynamic stability are considered for storage stability. It is observed that all the samples are extremely clear and stable without any precipitation or phase separation at ambient temperature for 90 days. The mean diameters of droplets measured by DLS altered negligibly after storage of 1 month (figure not shown). Thus, this study is evident for the stability of microemulsion at tested storage conditions.

Kinetic stability: The kinetic stability of microemulsion samples with same composition as mentioned for thermodynamic stability is performed by centrifugation at 2000 RPM for 15 min at ambient temperature (27 °C). We did not observe any phase separation (visual appearance) or change in the droplet size (DLS) for the above samples indicating their stability.

Encapsulation of Vitamin E (VE) in microemulsion

In this study, we used the microemulsion system for encapsulation of fat-soluble vitamin E which is a powerful biological antioxidant. Poor solubility of vitamin E in water or other inorganic solvents, poor stability to oxygen, light, and temperature and low or variable bioavailability limits its usage as functional ingredient in food and beverages[33]. Encapsulation of active agents like vitamins and antioxidants in a microemulsion system provides an opportunity for these active ingredients to be incorporated into beverages, food, or pharmaceutical products. Encapsulation leads to protection of active ingredients from oxidation

resulting in more stable active compounds which could have very prominent application in the food industry. Moreover, encapsulation of vitamin E in microemulsion system can offer nano-sized (<100nm) droplets causing enhanced stability, transmembrane passage across digestive tract and transdermal drug permeability enhancing its bioavailability. It also offers for controlled release and protection to encapsulated vitamin E as a delivery system[34,35]. A simple methodology is employed for the encapsulation of Vitamin E in the microemulsion. Primarily, vitamin E is mixed with oil phase (IPM) in various compositions. This mixture is then added to S_{mix} (Igepal CO-520/carbitol = 1:1). Then, water is further added to this mixture with continuous stirring on vortex at ambient temperature. The selected composition of microemulsion contains o\w structure so as to encompass oil soluble vitamin E in oily core of the microemulsion. All the microemulsion samples are then light-shielded to prevent the degradation and stored at 27 °C.

Encapsulation of Vitamin E in microemulsion: Figure 7 represents the UV-visible spectroscopy measurements of microemulsion (μE) samples without and with vitamin E to ensure the encapsulation of vitamin E in microemulsion droplets. It could be observed from the figure that (i) pure μE sample with composition 25 wt% S_{mix} (Igepal CO-520/ carbitol = 1:1), 5 wt% oil (IPM only) and 70 wt% water obtained λ_{max} at 290 nm, whereas, (ii) represents μE in the presence of vitamin E with composition 25 wt% S_{mix} (Igepal CO-520/carbitol = 1:1), 5 wt% oil (4% IPM + 1% VE) and 70 wt% water obtained λ_{max} at 300 nm. The peak obtained at 300 nm is the characteristic peak of vitamin E encapsulated in the microemulsion system. The absence of any other peaks indicated that Vitamin E is successfully encapsulated and there is no degradation of vitamin E into other oxidized components which otherwise would have given peaks at other wavelengths in UV-visible spectroscopy[32]. The absence of smooth curve in the UV-visible spectroscopy could be due to the presence of other components like surfactant and oil in the microemulsion system.

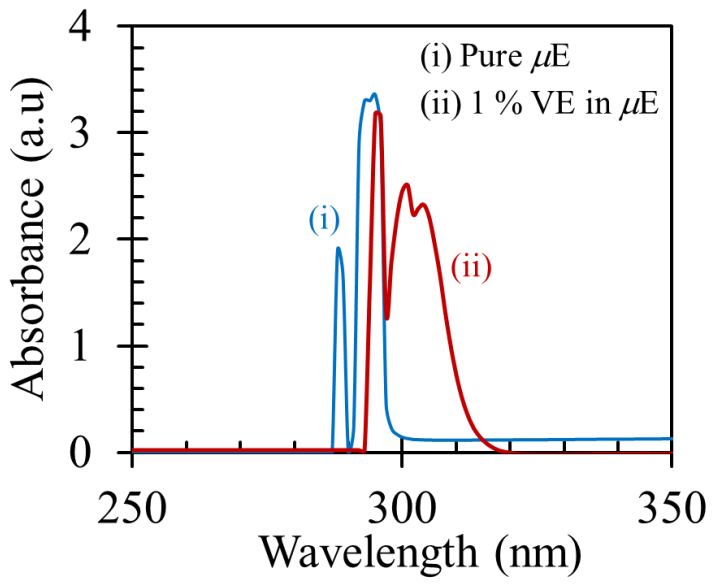
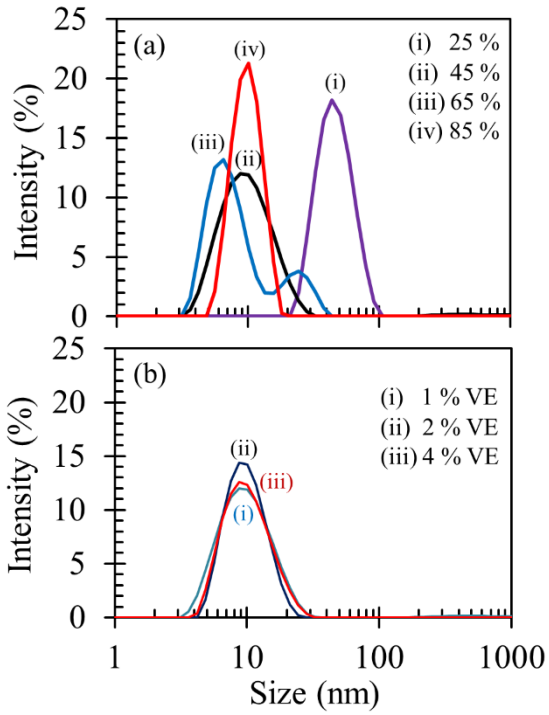


Figure 7: UV spectra of (i) Pure μE and (ii) 1% VE in μE with composition 25 wt% S_{mix} (Igepal CO-520/ carbitol = 1:1), 5 wt% oil and 70 wt% water at 27 °C.

Effect of surfactant concentration on encapsulation: To explore the effect of surfactant concentration on encapsulation of vitamin E, DLS studies are performed for the determination of sizes of encapsulated microemulsion samples. **Figure 8a** depicts the sizes of microemulsion samples with fixed concentration of vitamin E in 5 wt% oil (1 wt% vitamin E + 4 wt% IPM) and varying concentration of S_{mix} (Igepal CO-520/carbitol = 1:1) from 25–85 wt%. It could be observed that droplet size is almost similar in the range of 6.5–10 nm from 45–85 wt% of S_{mix} concentration representing that Vitamin E has been successfully encapsulated in microemulsion droplets of similar size. The slightly higher intensity at 85 wt% S_{mix} concentration could be due to the higher number density of microemulsion droplets. At 25 wt% of S_{mix}, the droplet size increased to 43.8 nm. This could be due to lower concentration of S_{mix} led to formation of larger size of microemulsion droplets where vitamin E is encapsulated. Also, the PDI of microemulsion samples is in the range of 0.2–0.4 indicating the uniformity in sizes of microemulsion samples. From these results, we could conclude that Vitamin E is successfully encapsulated in microemulsion samples with varying concentration of S_{mix} and the lower size of droplets contributed to enhanced stability of vitamin E encapsulated microemulsion samples.

Effect of Vitamin E concentration: The effect of vitamin E concentration on the size of microemulsion samples is also analyzed using DLS technique. Figure 8b represents the size of microemulsion samples with fixed concentration of 45 wt% S_{mix} with varying composition (1–4 wt%) of vitamin E in 5 wt% oil. This composition was selected due to smaller size of droplets obtained at lower concentration S_{mix} as shown in Figure. It is observed from the figure that the droplet sizes are around 8–10 nm for all the microemulsion samples with increasing concentration of vitamin E. This indicated the complete encapsulation of vitamin E in the microemulsion droplets. This could be due to formation stable o/w microemulsion droplets at a given composition which led to complete encapsulation of hydrophobic vitamin E in the core of o/w microemulsion droplets. And the PDI of 0.1–0.2 indicated uniformity in the sizes of the encapsulated vitamin E microemulsion samples.



Figures 8: Mean particle size of samples containing vitamin E and (Igepal CO-520/carbitol) S_{mix} = 1:1 (a) Effect of surfactant concentration in microemulsion containing 5 wt% oil (1 wt% vitamin E + 4 wt% IPM) (b) Effect of vitamin E concentration in microemulsion with composition 5 wt% oil + 45 wt% S_{mix}.

Conclusion

The novel reverse microemulsion is successfully prepared for the first time with a biocompatible nonionic surfactant Igepal CO-520 and nontoxic oils caprylic acid (CA), oleic acid (OA), isoamyl acetate (IAA) and isopropyl myristate (IPM). Igepal CO-520 forms clear and stable microemulsion with all the oils. However, greater microemulsion area is observed only with IPM in the presence of carbitol as co-surfactant. Electrical conductivity and viscosity measurements indicated the transitions from w/o microemulsion to o/w microemulsion through a bicontinuous phase with increasing water content. The microemulsion with Igepal CO-520 shows excellent wetting and spreading ability along with good thermodynamic, kinetic and storage stability. The microemulsions also offers an excellent scope for encapsulation of vitamin E due to the absence of charge on the surfactant hydrophilic head group. The results obtained from this research might have potential applications in designing microemulsions for cosmetic, food and pharmaceutical applications.

Acknowledgements

DV thank Navrachana University for Seed Grant (NUV/Seed Grant/2023_24/02).

References

1. McClements, D. J. (2012). Nanoemulsions versus microemulsions: terminology, differences, and similarities, *Soft Matter* 8 (6) 1719-1729. https://doi.org/10.1039/c2sm06903b
2. Klossek, M. L. Touraud, D. Kunz, W. (2012). Microemulsions with renewable feedstock oils, *Green Chemistry* 14 (7) 2017-2023. https://doi.org/10.1039/C2GC35035A
3. Ryan, L. D., Schubert, K. V., & Kaler, E. W. (1997). Phase Behavior of Microemulsions Made with n-Alkyl Monoglucosides and n-Alkyl Polyglycol Ethers. *Langmuir*, *13*(6), 1510–1518. https://doi.org/10.1021/la9608730
4. Monzer Fanun. (2008) *Microemulsions*: *properties and applications*, CRC press.
5. YT. Zhang, JH. Zhao, SJ. Zhang, YZ. Zhong, Z. Wang, Y. Liu, F. Shi, NP. Feng, Enhanced transdermal delivery of evodiamine and rutaecarpine using microemulsion, Int. J. Nanomed. 6 (2011) 2469-2482. https://doi.org/10.2147/ijn.s25258)

6. Shishoo, C., Chudasama, A., Patel, V., Nivsarkar, M., & Vasu, K. (2011). Investigation of microemulsion system for transdermal delivery of itraconazole. *Journal of Advanced Pharmaceutical Technology & Research, 2*(1), 30. https://doi.org/10.4103/2231-4040.79802
7. Tsai, Y.-H., Fu, L.-T., Huang, C.-T., Chang, J.-S., Huang, Y.-B., & Wu, P.-C. (2011). Formulation Optimization of Estradiol Microemulsion Using Response Surface Methodology. *Journal of Pharmaceutical Sciences, 100*(10), 4383–4389. https://doi.org/10.1002/jps.22600
8. Deutch-Kolevzon, R., Aserin, A., & Nissim Garti. (2011). Synergistic cosolubilization of omega-3 fatty acid esters and CoQ10 in dilutable microemulsions. *Chemistry and Physics of Lipids, 164*(7), 654–663. https://doi.org/10.1016/j.chemphyslip.2011.06.010
9. Nissim Garti, Anan Yaghmur, Leser, M. E., Clement, V., & Watzke, H. (2001). Improved Oil Solubilization in Oil/Water Food Grade Microemulsions in the Presence of Polyols and Ethanol. 49(5), 2552–2562. https://doi.org/10.1021/jf001390b
10. Zhong, F., Yu, M., Luo, C., Shoemaker, C. B., Li, Y., Xia, S., & Ma, J. (2009). Formation and characterisation of mint oil/S and CS/water microemulsions. *Food Chemistry*, *115*(2), 539–544. https://doi.org/10.1016/j.foodchem.2008.12.048
11. Engelskirchen, S., Maurer, R., Levy, T., Berghaus, R., Helmut Auweter, & Glatter, O. (2012). Highly concentrated emulsified microemulsions as solvent-free plant protection formulations. *Journal of Colloid and Interface Science, 388*(1), 151–161. https://doi.org/10.1016/j.jcis.2012.06.084
12. Tamhane, V. A., Dhaware, D. G., Khandelwal, N., Giri, A. P., & Venkateswarlu Panchagnula. (2012). Enhanced permeation, leaf retention, and plant protease inhibitor activity with bicontinuous microemulsions. *Journal of Colloid and Interface Science, 383*(1), 177–183. https://doi.org/10.1016/j.jcis.2012.06.025
13. Chin, C.-P., John Chi-Wei Lan, & Wu, H. (2012). Study on the Performance of Lambda Cyhalothrin Microemulsion with Biodiesel as an Alternative Solvent. *Industrial & Engineering Chemistry Research, 51*(12), 4710–4718. https://doi.org/10.1021/ie201151p
14. Acosta, E. (2009). Bioavailability of nanoparticles in nutrient and nutraceutical delivery. *Current Opinion in Colloid & Interface Science, 14*(1), 3–15. https://doi.org/10.1016/j.cocis.2008.01.002
15. Garti, N., Yaghmur, A., Aserin, A., Spernath, A., Elfakess, R., & Ezrahi, S. (2003). Solubilization of active molecules in microemulsions for improved environmental protection. *Colloids and Surfaces*

A: Physicochemical and Engineering Aspects, 230(1-3), 183-190. https://doi.org/10.1016/j.colsurfa.2003.09.020

16. Soumya Sundar Mati, Tapas Kumar Mondal, Dhar, S., Chall, S., & Subhash Chandra Bhattacharya. (2012). Differential contribution of Igepal and CnTAB micelles on the photophysics of nonsteroidal drug Naproxen. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 92,* 122–130. https://doi.org/10.1016/j.saa.2012.02.042
17. Paul, B. K., & Rajib Kumar Mitra. (2005). Water solubilization capacity of mixed reverse micelles: Effect of surfactant component, the nature of the oil, and electrolyte concentration. *Journal of Colloid and Interface Science, 288*(1), 261–279. https://doi.org/10.1016/j.jcis.2005.02.088
18. Frassinetti, S., Barberio, C., Caltavuturo, L., Fava, F., & Di Gioia, D. (2011). Genotoxicity of 4-nonylphenol and nonylphenol ethoxylate mixtures by the use of Saccharomyces cerevisiae D7 mutation assay and use of this text to evaluate the efficiency of biodegradation treatments. E*cotoxicology and Environmental Safety, 74*(3), 253–258. https://doi.org/10.1016/j.ecoenv.2010.10.039
19. van, Panman, M. R., Lotze, S., Zhang, J., Vad, T., Bakker, H. J., Wiebke, & Sander Woutersen. (2012). Structure and dynamics of water in nonionic reverse micelles: A combined time-resolved infrared and small angle x-ray scattering study. *JuSER (Forschungszentrum Jülich), 137*(4). https://doi.org/10.1063/1.4736562
20. Richard, B. Lemyre, J. L. Ritcey, A. M. (2017) Nanoparticle size control in microemulsion synthesis, *Langmuir, 33* (19) 4748-4757. https://doi.org/10.1021/acs.langmuir.7b00773
21. Zhang, M., Cushing, B. L., & O'Connor, C. J. (2008). Synthesis and characterization of monodisperse ultra-thin silica-coated magnetic nanoparticles. *Nanotechnology, 19*(8), 085601. https://doi.org/10.1088/0957-4484/19/8/085601
22. Napaporn Komesvarakul, Sanders, M. D., Szekeres, E., Acosta, E. J., Faller, J. F., Mentlik, T., Fisher, L. B., Nicoll, G., Sabatini, D. A., & Scamehorn, J. F. (2006). Microemulsions of triglyceride-based oils: The effect of co-oil and salinity on phase diagrams. *PubMed, 57*(4), 309–325
23. Zhang, Y., Zhang, X. Y., Chai, J. L., Cui, X. C., Pan, J., Song, J. W., Sun, B., & Lu, J. J. (2017). The phase behavior and solubilization of isopropyl myristate in microemulsions containing hexadecyl trimethyl ammonium bromide and sodium dodecyl sulfate. *Journal of Molecular Liquids,* 244, 262–268. https://doi.org/10.1016/j.molliq.2017.08.074

24. Syed, H. K., & Peh, K. K. (2014). Identification of phases of various oil, surfactant/ co-surfactants and water system by ternary phase diagram. *PubMed*, *71*(2), 301–309
25. Nandi, I., Bari, M., & Joshi, H. (2003). Study of isopropyl myristate microemulsion systems containing cyclodextrins to improve the solubility of 2 model hydrophobic drugs. *AAPS PharmSciTech*, *4*(1), 71–79. https://doi.org/10.1208/pt040110
26. Li, Y., Wang, C., Tao, Z., Zhao, Z.-G., You, L., Zheng, R., Guo, X., & Zhang, Z. (2019). Enhanced Antioxidant and Antiproliferative Activities of Cymbopogon citratus *(DC.) Stapf Essential Oils in Microemulsion. 7*(18), 15173–15181. https://doi.org/10.1021/acssuschemeng.9b01606
27. Daicic, J., Olsson, U., & Wennerstroem, H. (1995). Phase Equilibria of Balanced Microemulsions. *Langmuir*, *11*(7), 2451–2458. https://doi.org/10.1021/la00007a023
28. Gradzielski, M., Duvail, M., Malo, P., Simon, M., Talmon, Y., & Zemb, T. (2021). Using Microemulsions: Formulation Based on Knowledge of Their Mesostructure. *Chemical Reviews*, *121*(10), 5671–5740. https://doi.org/10.1021/acs.chemrev.0c00812
29. Wang, W., Wei, H., Du, Z., Tai, X., & Wang, G. (2015). Formation and Characterization of Fully Dilutable Microemulsion with Fatty Acid Methyl Esters as Oil Phase. *ACS Sustainable Chemistry & Engineering*, *3*(3), 443–450. https://doi.org/10.1021/sc500667n
30. Nazar, M. F., Saleem, M. A., Bajwa, S. N., Yameen, B., Ashfaq, M., Zafar, M. N., & Zubair, M. (2017). Encapsulation of Antibiotic Levofloxacin in Biocompatible Microemulsion Formulation: Insights from Microstructure Analysis. *The Journal of Physical Chemistry B*, *121*(2), 437–443. https://doi.org/10.1021/acs.jpcb.6b09326
31. Santos, Santos, G. S., Thallysson Carvalho Barbosa, Souza, L., Menezes, L. G., Luciana Nalone Andrade, Bani, C., Hugo, V., de, R., Aleksandra Zielińska, Marcelo, Juliana Cordeiro Cardoso, Santini, A., Souto, E. B., & Severino, P. (2021). Citrus sinensis Essential Oil-Based Microemulsions: Green Synthesis, Characterization, and Antibacterial and Larvicide Activities. *ACS Food Science & Technology*, *1*(3), 462–469. https://doi.org/10.1021/acsfoodscitech.0c00150
32. Chintya Gunarto, Ju, Y.-H., Jindrayani Nyoo Putro, Phuong Lan Tran-Nguyen, Felycia Edi Soetaredjo, Shella Permatasari Santoso, Aning Ayucitra, Artik Elisa Angkawijaya, & Suryadi Ismadji. (2020). Effect of a Nonionic Surfactant on the Pseudoternary Phase Diagram and Stability of Microemulsion. *Journal of Chemical & Engineering Data*, *65*(8), 4024–4033. https://doi.org/10.1021/acs.jced.0c00341

33. M. Ali Aboudzadeh, Ehsan Mehravar, Fernández, M., Lezama, L., & Radmila Tomovská. (2018). Low-Energy Encapsulation of α-Tocopherol Using Fully Food Grade Oil-in-Water Microemulsions. *ACS Omega*, *3*(9), 10999–11008. https://doi.org/10.1021/acsomega.8b01272
34. Feng, J., Wang, Z., Zhang, J., Wang, Z., & Li, F. (2009). Study on food-grade vitamin E microemulsions based on nonionic emulsifiers. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 339*(1-3), 1–6. https://doi.org/10.1016/j.colsurfa.2009.01.002
35. Saberi, A. H., Fang, Y., & McClements, D. J. (2013). Fabrication of vitamin E-enriched nanoemulsions: Factors affecting particle size using spontaneous emulsification. *Journal of Colloid and Interface Science, 391*, 95–102. https://doi.org/10.1016/j.jcis.2012.08.069

Glossary

Surfactant- Surfactants are chemical compounds that decrease the surface tension or interfacial tension between two liquids, a liquid and a gas, or a liquid and a solid.

Co-surfactant- A chemical substance that is used (small amount) in addition to a surfactant to improve its performance, especially a second surfactant that is used in conjunction with a primary surfactant.

Microemulsion- Microemulsions are clear, thermodynamically stable isotropic liquid mixtures of oil, water and surfactant, frequently in combination with a cosurfactant.

Phase Diagram- phase diagram, graph showing the limiting conditions for solid, liquid, and gaseous phases of a single substance or of a mixture of substances while undergoing changes in concentration at fixed temperature.

Psuedoternary Phase diagram- A pseudoternary phase diagram is a tool that optimizes the three components of any typical microemulsion or emulsion, i.e., water, oil, and surfactant, to obtain the concentration range of these components, which form a stable emulsion.

Nanoparticle- A nanoparticle or ultrafine particle is usually defined as a particle of matter that is between 1 and 100 nanometres (nm) in diameter.

Microstructures- Microstructure are material structures seen at the micro level.

Thermodynamic Stability- Thermodynamic stability occurs when a system is in its lowest energy state, or in chemical equilibrium with its environment.

Vitamin E- Vitamin E is a nutrient that's important to vision, reproduction, and the health of your blood, brain and skin.