Ultrasound-assisted synthesis and characterization of sesame oil based nanoemulsion

Kumar Namratha Vinaya¹, Ann Mary John¹,³, Mona Mangsatabam¹ and Mercy Anna Philip²

¹Department of Chemical Engineering, Government Engineering College, Thrissur, Kerala, India. PIN-680009
²Assistant Professor, Department of Chemical Engineering, Government Engineering College, Thrissur, Kerala, India. PIN-680009
³E-mail:annmaryjohn97@gmail.com

Abstract. Nanoemulsions are emulsions having droplet sizes in the nanometre range. Nanoemulsions have unique properties such as robust stability, transparent nature, high surface area, and tunable rheology. The present study focuses on the synthesis of sesame oil-based nanoemulsion using ultrasonic emulsification to break down particle size to nanometric range. The method uses small surfactant concentration and low energy to synthesize homogenous nanoemulsion. The emulsion is stabilized using Tween 20 surfactant. Aqueous nanoemulsion samples were prepared using sesame oil by varying the surfactant concentration to determine the most stable composition, and different properties of each sample prepared, were analyzed. The ultrasonication time was also varied to study the impact of sonication time. Nanoemulsions formed were then evaluated for their various properties. The droplet size and zeta potential for all samples were determined by the Dynamic Light Scattering technique wherein the best nanometer size distribution was found to be at 1:3 (oil: surfactant) composition. The physical stability was determined using a centrifuge at 3000 rpm. Conductivity, pH, and density of the samples were then measured. A polydispersity index of 0.514 was obtained for the sample of oil: surfactant ratio 1:3. Fourier Transform Infrared Spectroscopy studies were conducted for all samples.

1. Introduction
Nanoemulsions or sub-micron emulsions are emulsions of droplet sizes in the nanometre range, typically varying between 1nm and 1000 nm. A nanoemulsion may be classified as oil-in-water, water-in-oil, or bi-continuous depending on the dispersed phase and the dispersion medium. The dispersed phase is stabilized by an interfacial layer of surfactant that helps by reducing the surface tension between the two immiscible liquids. A nanoemulsion is generally characterized by its stability and clarity [1]. The droplet size in a nanoemulsion is found to be less than 25% of the wavelength of visible light. As a result, nanoemulsions are generally transparent. The methods that are used for the formation of nanoemulsion employ energy in the form of heat, light, or sound to tear the droplets into the nano-size range.

Science behind the formation of nano emulsions
The science behind the formation of nanoemulsion is to be understood in order to control the size of droplets. A macro emulsion is formed first which is then converted to a nanoemulsion. Multiphase
dispersions constitute a continuous phase, and a dispersed phase of particles up to nanometre sizes. The density difference between the two phases is small such that gravitational sedimentation can be prevented for a long time. Structural transitions may occur in these dispersions if the dispersed phase possesses a higher volume fraction. These nanoscale colloids can be stabilized by different mechanisms. The suspension will remain homogenous if there are repulsive interactions between colloids that hinder particles from flocculating. If the interactions are attractive, rapid sedimentation and flocculation occur [2].

An interfacial tension exists between the two liquid phases where ever they are in contact because of the differences in attractive interactions between molecules of both the liquids [3]. Surfactants or amphiphilic surface active molecules are added to decrease the interfacial tension. These surfactants are extremely soluble in either one of the liquid phases. The upper liquid phase which is a hydrocarbon oil has a smaller mass density than the lower liquid phase which is water. The system changes back to the lowest energy configuration at thermodynamic equilibrium in case of the absence of surfactants or other impurities for an extended period of time.

Methods of preparation of nanoemulsions
Nanoemulsions are non-equilibrium systems and therefore their formulation involves either a huge amount of energy or surfactants input and sometimes a combination of both. There are a number of methods developed for nanoemulsion formulation [4].

1.1.1. High energy methods. The oil-water phases are broken down to nano particles using mechanical devices which generate high disruptive forces in these methods.

High pressure homogenization: It produces nanoemulsions of low particle size which is of 10-100 nm. The oil and water mixture is passed through a small inlet orifice at high pressures of the range of 500-5000 psi to achieve dispersion of it. The hydraulic shear and intense turbulence that the product is subjected to create extremely fine particles of the emulsion.

Ultrasonicators: In this technique, the pre-mixed emulsion is subjected to a high ultrasound frequency which reduces the droplet sizes to nano range which is then, for uniform size distribution, taken through a region of high shear. The energy for ultrasonic emulsification is provided by a sonicator probe which has a piezoelectric quartz crystal that produces vibrations when the tip of the sonicator comes in contact with the liquid. This results in cavitation and in turn the release of energy that breaks down the particles to nanometer size because of the disintegration of vapor cavities within the liquid.

Microfluidization: The product, in this method, is forced into an interaction chamber which uses a positive displacement pump of the pressure of 500 to 20000 psi to form sub-micron elements in the micro channels to the impingement area.

1.1.2. Low energy methods. Phase inversion temperature: Preparation of nanoemulsion using this method involves phase transitions by changing either the composition or temperature while the other parameter is kept constant. The formation of fine particles here does not require an outside force.

Spontaneous emulsification: The preparation of nanoemulsion by this method happens in three stages. First, the organic solution which consists of oil, hydrophilic and lipophilic surfactants, and water miscible solvent is formed. Secondly, by magnetic stirring, the organic phase is injected into the aqueous phase to form O/W (Oil dispersed in water) emulsion. The organic solvent is then evaporated off in the third stage.

Hydrogel method: In the hydrogel method, the solvent is evaporated after the drug solution is made and emulsified into the other liquid which precipitates the drug. Stirrers of high speed are used for control of crystal growth.

Properties of nanoemulsions
The reduction of droplet size of the emulsion to nanoscale has led to some interesting changes in the properties of the emulsion. Due to their small droplet sizes, nanoemulsions have many advantages over conventional emulsions [5].
1.1.3. **Thermodynamic stability.** The long-term physical stability is a direct result of small droplet size. They do not separate against gravity nor do the droplets aggregate. The encapsulated substances have amplified bioavailability which makes them demanded in food applications.

1.1.4. **Transparency.** They have high optical transparency due to the very small size of nanoparticles. The transparency of nanoemulsions is highly sought after because of weak light scattering of the final products made.

1.1.5. **High surface area.** The small size of the droplets provides a greater surface area of absorption for the drugs. The minute droplet sizes and the ability to solubilize hydrophobic drugs increase bioavailability and significantly the drug dissolution rate. The large surface area also influences the drug transport positively by targeting them to specific sites.

1.1.6. **Tunable rheology.** They possess unusual elastic properties due to the nanoscale size. As a result, these are of great value in the field of nanomaterials [6]. The rheological properties are tuned by adding salt and depletion agents and with the right droplet size and dispersed phase volume fraction.

**Objective of the experiment**

The objective of the experiment is to synthesize a sesame oil-in-water nanoemulsion stabilized by the surfactant Tween 20 and to prepare different samples with varying concentrations of oil and surfactant using the high energy ultrasonication technique, with each sample ultrasonicated for three different time durations. The effects of time and concentration of ultrasonication on the stability of nanoemulsion is studied by testing the prepared samples for various properties like pH, conductivity, density, transmittance, droplet size, zeta potential, and physical stability. The results obtained are compared with previous studies for identifying the best sample concentration that exhibits the desired properties and the required stability.

1.1.7. **Advantages of using sesame oil.** Sesame oil is known to significantly reduce the risk of coronary heart disease (CHD) as it contains high unsaturated fatty acid, especially polyunsaturated fatty acids (PUFAs). It contains significant vitamins and minerals such as copper, zinc, magnesium, iron, and calcium. The oil also has ‘lignans’ which is credited to have unique chemical and physiological properties, and known for being a powerful antioxidant. Sesame oil can also lower blood pressure and has antibacterial and anti-inflammatory properties. Tyrosine, the amino acid it contains is a direct influence on serotonin activity.

1.1.8. **Selection of surfactant-Tween 20.** Polyoxyethylene (20) sorbitan monolaurate, known by its market name as TWEEN 20, has an HLB (hydrophilic-lipophilic balance) value of 16.5 and is 84% hydrophilic. It is an excellent O/W emulsifier. It has excellent stability and is relatively nontoxic, making it a popular surfactant in many medical formulations. Tween 20 is a polyoxyethylene sorbitol ester, containing approximately 20 units of ethylene oxide per unit of sorbitol. Tween 20 is biocompatible and is not affected by changes in pH and is generally regarded safe. It reduces the energy required for the formation of nanoemulsion, consequently improving the stability. Studies also show that oil is better solubilized in Tween 20 compared to other non-ionic surfactants.

2. **Experiment**

2.1. **Preparation of nanoemulsion**

A nanoemulsion is formed from an aqueous phase and an internal phase. The internal phase consists of the oil and the surfactant which are mixed in different proportions. Initially, the mixture is blended in the magnetic stirrer at 540 rpm for about 3 minutes to get uniform distribution and dissolution of surfactants in the mixture.

In order to obtain oil in water nanoemulsion, i.e. to get oil droplets dispersed in the aqueous phase, distilled water was added drop-wise in the internal phase mixture and made up to a volume of 30 ml. The emulsion was stirred using a magnetic stirrer for about 10 minutes to get uniform distribution, after which 1:1, 1:1.5, 1:2, 1:3, 1:4 and 1:5 oil to surfactant compositions were prepared. The
emulsions were then sonicated in the ultrasonicator bath of frequency 20 kHz for 10, 20, 30 minutes respectively for each composition.

2.2. Characterization

2.2.1. pH and conductivity measurement. pH was measured using a pH meter at 25°C temperature. For nanoemulsions, pH values ranging from 4.2 to 5.8 are generally observed making them non-irritant in nature for pharmacological applications. Emulsions with vegetable oil might have a decreased pH as fatty acid esters get hydrolysed to free fatty acid degradation products. Electrical conductivity measurements were performed for finding the nature of the aqueous phase and to identify phase inversion. O/W nanoemulsions have high conductivity as their continuous phase is water whereas W/O (water dispersed in oil) nanoemulsions have low conductivity.

2.2.2. Droplet size and PI measurement. Droplet size and Polydispersity Index (PI) are measured using the DLS (Dynamic Light Scattering) technique. The experiment was performed on the Horiba Scientific nanoparticle analyzer. For measuring the droplet size accurately, the samples must be transparent and suitably diluted for dynamic light scattering. The test was done at a temperature of 25.1°C with a scattering angle of 173°. The sesame oil has a refractive index of 1.47 which determines the light scattering properties of the oil. The quality of droplet size distribution is measured using PI which is a dimensionless number having a range of 0.1-1. A 0.4 or below PI means the droplets are extremely monodispersing and greater than 0.7 indicates a broad droplet size distribution range.

2.2.3. Physical stability measurements. The physical stability is checked by centrifuging the samples at 3000 rpm for 10 minutes. The samples that did not separate were further centrifuged for 5 more minutes. The samples that showed considerable stability and other desired properties are taken for further characterization.

2.2.4. Zeta potential. It is the difference in potential between mobile and the stationary layers of the dispersion medium. pH, ionic strength, concentration are the factors that determine the zeta potential which is an indicator of dispersion stability [7]. Emulsions with high zeta potential (-41 to -50 mV) are electrically stable and emulsions with low values (-11 to -20 mV) tend to coagulate, possibly leading to poor physical stability. The relative strengths of the attractive and repulsive forces determine the stability of a system.

2.2.5. Fourier transform infrared spectroscopy (FTIR)

Functional groups are determined using FTIR. A molecule existing in ground state at low temperature gets excited to higher energy levels on absorbing radiant energy. The energy difference between ground and excited states are then determined by IR spectroscopy. It is also carried out for assessing the interactions between the drug and the nanoemulsion, to find crosslinking, polymerization, and assessment of drug loading.

3. Results

pH and conductivity of the precursors were measured, and it was found that both oil and surfactant showed a very low conductivity and the components were basic in nature. The pH of oil was found to be 8.4 and that of Tween 20 was 7.5 while the conductivity of oil was found to be 0.190 μS and that of Tween 20 was 2.410 μS.

Viscosity of sesame oil is 0.0388 Pas and that of Tween 20 is 0.250 Pas. The compositions of the nanoemulsions were calculated on the basis of the volume fraction of oil used as;

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\text{Composition} = \frac{\text{Volume of oil}}{\text{Volume of oil} + \text{Volume of surfactant}}
\] (1)

3.1. Particle Size

The particle sizes obtained for the samples using Dynamic Light Scattering technique was plotted on a graph to show the variation at different compositions ultra-sonicated for 20 minutes. It was seen that the particle size seemed to decrease with surfactant concentration to reach a minimum after which it
showed a drastic rise. Therefore, it can be concluded that the best size range is obtained at the composition of 1:3. At low surfactant concentration, the surfactant molecules cannot cover the oil molecules. Eventually, the oil droplets coalesce and agglomerate.

Figure 1. Plot of particle size with varying composition (v/V)

When the particle size distribution vs., ultrasonication time was plotted for various compositions, it was seen that the nanoemulsions formed were in the desired size range of 100-500 nm when ultrasonicated for 20 minutes. The increase of particle size after 20 minutes is because of agglomeration of particles in that size range owing to the development of weak attractive forces between the particles.

Figure 2. Plot of particle size distributions with varying sonication time for all compositions

3.2. Density
Density for various compositions were measured and found to be in the range of 900-1000 kg/m$^3$ which proved that the formed nanoemulsions were not denser than water. Varying the ultrasonication time while keeping the composition constant did not make any marked difference to density as the composition was essentially the same throughout the sonication time.

3.3. pH analysis
It was seen that the nanoemulsions formed were non-irritant in character owing to a pH range of 4.5-5.1, which is in fact the pH of distilled water. The variation in pH was then plotted which showed a decrease in pH with composition. Observation of ultrasonication time to pH of the nanoemulsion indicated that the desired pH was obtained at 20 minutes for most of the compositions.
3.4. Conductivity
The electrical conductivity of a nanoemulsion will determine whether the prepared nanoemulsion is O/W or W/O. The results obtained from the experiments showed a high conductivity which meant O/W emulsions were formed. Relatively high conductivities of 183μS were seen in compositions of 1:5 to 1:3. Varying the ultrasonication time did not create any change to the conductivity for a particular composition.

3.5. Polydispersity index
A highly polydisperse emulsion has a high variation in droplet size. Because of such variations, the most common phenomenon that occurs in nanoemulsions is Oswald Ripening which eventually causes destabilization of nanoemulsions. Smaller particles withstand gravitational separation, aggregation, amalgamation, and creaming due to Brownian motion. Lower PI signifies higher stability. For sample 1:3, the PI was found to be the lowest at 0.514, followed by a PI of 0.555 for sample 1:2.

3.6. Zeta potential
The plots of zeta potential obtained from the DLS technique for each of the compositions are shown below. The x-axis value when the intensity is at the maximum gives the zeta potential for that composition. The zeta potentials are plotted as shown for all samples at 20 minutes of ultrasonication time. It can be seen that 1:4, 1:3, and 1:2 showed high zeta potential value thereby indicating its colloidal stability to be moderate.
FTIR tests were conducted for all samples to identify and assess the polymerization and crosslinking in each sample and the functional groups they contain and their path of attachment in future drug loading purposes. The wavenumbers corresponding to different functional groups are obtained from the IR spectrum table by ‘Sigma-Aldrich’. The range indicating the –OH group in the nanoemulsion is from 3300-3500 cm⁻¹. The wavenumber ranges from 1620-1670 cm⁻¹ denotes the presence of –C=C- bond which is the functional group present in the Polysorbate molecule. The indication of –C-O- bond at the range of 1050-1200 cm⁻¹ shows the non-deteriorated molecules of oil particles. The combined graphs of all FTIR analysis showed a similar variation percentage transmittance with wavenumber proving that all samples prepared had the same functional groups and orientation.

![FTIR results of different samples ultrasonicated for 20min](image)

3.8. Centrifugation
Centrifugation was done for all samples to determine physical stability with time and environmental conditions. The samples were subjected to centrifugation at 3000 rpm and for 10 minutes where it was seen that the sample compositions of 1:5, 1:4, and 1:3 did not get separated into two layers. This showed that they were stable under those conditions. The 1:2 sample got separated after centrifuging for 10 minutes which indicated its unstable nature.

4. Conclusions
In past years, nanoemulsions have gained much interest for their widespread application in food, cosmetics, and pharmaceuticals, among others. A unique combination of excellent properties like high surface area to volume ratio, exceptional stability as well as low levels of toxicity gives nanoemulsion an upper hand as a novel drug delivery system. With the help of a suitable surfactant, a nanoemulsion of sesame oil in water, with considerable stability and other desirable properties can be prepared by selecting the appropriate conditions during its synthesis. The oil to surfactant ratio and the time of ultrasonication play an important role in determining its droplet size and physical stability. Ultrasonication time of the present experiment of sesame oil nanoemulsion greatly affected the properties of a sample composition as the stability increased with increasing surfactant concentration. The optimum ratio was found to be 1:3 which was ultrasonicated for 20 minutes to give a mean particle size of 189.5 nm. From further studies, it was seen that the zeta potential for 1:4, 1:3, and 1:2 showed high values, hence proving their stability. Centrifugation at 3000 rpm performed for 10 minutes also indicated a considerably better stability for the same compositions over others. FTIR results further showed that there was no deterioration or disarrangement of functional groups due to ultrasonication. pH and conductivity studies were performed to determine that the said nanoemulsions were in the desirable ranges of these properties and therefore was non-irritant, non-toxic, and formed O/W nanoemulsions.
Determining the interactions of the prepared nanoemulsion at 1:3 and 1:4 with various drugs is the next step to achieving targeted drug delivery. The corresponding drug-loaded nanoemulsion will be subjected to FTIR analysis to measure the stability and the drug loading characteristics within the nanoemulsion.

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