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Evaluation of emulsification kinetics of oil in water

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Abstract

Emulsions are the complex fluids and their stability depends, or is reflected by the size of the emulsion droplets which is considered to be critical. In the design and study of an emulsification process, knowledge of factors governing the particle size and size distribution of the droplets is an important consideration. The study of kinetics of emulsification involves various parameters and the droplet sizing is a significant one. In this work the effects of various experimental factors has been carried out to study the kinetics of emulsification of oil in water (e.g. agitation duration, agitation rate, surfactant concentration, oil concentration, ageing). Sizing of droplets was carried out by Phase Doppler Anemometry (PDA), Photo Correlation Spectroscopy (PCS), Laser Diffraction (LD) and Microscopy. The effect of these parameters during the emulsification process was studied and the reason for imparting significance on these parameters is for the fact that controlling these critical parameters can assist to obtain a desired final product as this paves the path for achieving the quality aspect of being "Right at the first time" which is a central issue in Quality by Design (QbD). The comparison of size distribution obtained by these sizing methods was studied. Results have shown that by increasing agitation duration and rate a decrease in droplet diameter was observed, surfactant concentration cause a decrease in droplet diameter and an increase in oil concentration increased the droplet diameter. Ageing has also shown an increase in droplet diameter. Regarding the techniques considered, PDA was limited by the imposed dilution on emulsions to allow measurements, PCS was used at the marginal upper limit range thus care had been taken to present accurate measurements, LD was sensitive to dilution of samples prior to measurements and Microscopy, a tedious technique difficult to use and when limited samples are used one may have non representative results. The possibility of using Phase Doppler Anemometry (PDA) as online monitoring equipment (as a Process Analytical Technology) was discussed where the emulsion droplets could be assessed online there by contributing to the real time emulsification control was discussed. The study of viscosity of these emulsions was done as this is one of the significant criteria for the quality control aspects. From the techniques used, PDA seems to be most adequate as online/inline equipment for monitoring the process of emulsification.

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Chapter 1 INTRODUCTION

Emulsification is a key technology in pharmaceutical, cosmetic and food industries. It allows the ‘mixing’ of non mixable liquids in a thermodynamic unstable system. Emulsions stability is considered a primary requirement for a wide variety of the many industrial applications of emulsions. They are the complex fluids that constitute the broad classes of materials whose physical behavior cannot be explained from the chemical constituents alone, without taking into account the characteristics and organization of the structures making up the bulk fluid. Emulsion quality rely on the stability factor of the emulsion [1] and emulsion rheology and stability are closely related both depending on several parameters discussed later.

1.1 Basic aspects of Emulsions

An emulsion is a dispersion of a liquid phase in another non-miscible liquid phase. This dispersion is usually not thermodynamically stable and evolves toward the state of minimum energy, which is the complete separation of the two phases. One of these two components is present in the form of finely dispersed spherical droplets in the second, continuous phase. The presence of surfactant retards the destabilizing process and ensures long-term stability. An emulsified state may exist in almost every liquid-liquid system where miscibility is not complete, as step in a process or as a final product. This explains the major interest of emulsions in chemical, food and petroleum industries. The International Union of Pure and Applied Chemistry (IUPAC) define “Emulsion” as “in an emulsion liquid droplets and/or liquid crystals are dispersed in a liquid. According to European Pharmacopoeia “*liquid oral solutions, emulsions or suspensions containing one or more active ingredients in a suitable vehicle, some liquids for oral administration may consist of liquid active ingredients as such. These preparations may contain suitable antimicrobial preservatives, anti oxidants and other excipients such as dispersing, suspending, thickening, emulsifying, buffering, wetting, competent authority* [2].

The most common types of pharmaceutical emulsions include water as one of the phases and oil as the other. Energy is required to increase the surface between the water and oil and this energy is called surface free energy. In other words it can be put as the interfacial free energy, the minimum amount of work required to create the interface.

This energy is the surface energy which we measure when we determine the interfacial tension between two phases. Interfacial tension can be explained as the measure of the difference in nature of the two phases meeting at the interface, greater the dissimilarity in their nature, the greater the interfacial tension. Expansion of an interface is the minimum work *(W_{min})* utilized to create the additional amount of that interface is the product of the interfacial tension (γ_I) and the increase in area of the interface which can be given by the following equation:

$$W_{min} = \gamma_1 \Delta \qquad \textbf{\textit{Equation}}\ (1)$$

1.1.1 Simple emulsions

Oil in water (o/w) emulsion consists of oil droplets dispersed in water and water in oil emulsion (w/o) consists of water droplets dispersed in oil. The type of emulsifier used is a decisive factor in the type of emulsion formed, o/w or w/o. Low molecular weight; hydrophilic surfactants induce formation of o/w emulsions, lipophilic surfactants favor *w/o* emulsions. Use of water-soluble macromolecular surfactants also results in formation of *o/w* emulsions. This generalization is known as *Bancroft's Rule,* which can be explained as follows: the phase in which the surfactant is more soluble is the outer phase. As the boundary film has two surface tensions, we may also say: the film curves in towards the side with the higher γ value, that is, the disperse phase are the one with the higher γ [3]. According to oriented wedge theory the type of emulsion can be explained as follows. The part of the surfactant molecule that has the greater cross-sectional area is oriented towards the dispersion medium. This maximizes the density of the interfacial film of the emulsifier.

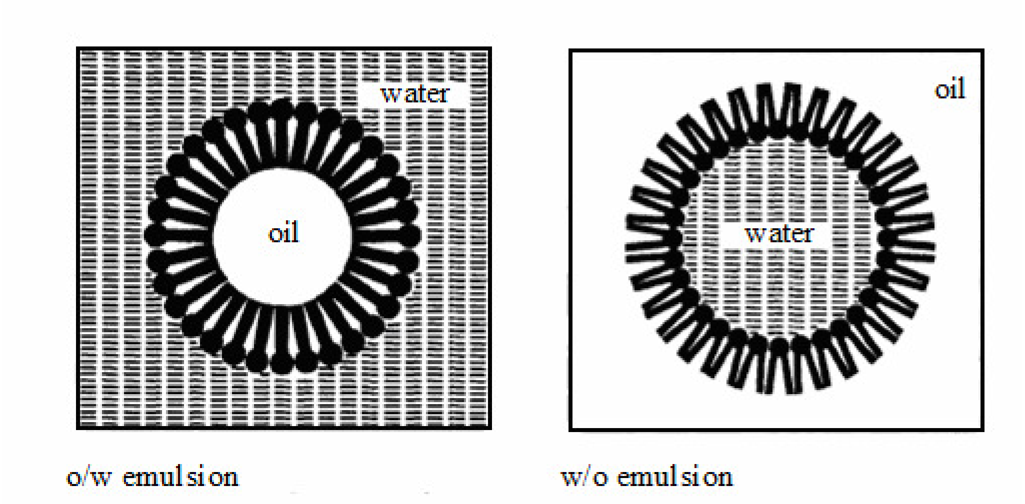


Figure 1. Types of simple emulsion.

According to Ostwald theory the ratio between the phases contribute to the type of the emulsion formed, for instance at a value of dispersed phase volume $\phi > 0.74$, an emulsion is too densely packed and either phase inversion or disruption of the emulsion will occur. For a given system, the phase volume ratios between 0.26 and 0.74 can contribute to both o/w and w/o emulsions are possible; below and above these ratios, only one type can exist if the spheres or the droplets were homogeneous [3].

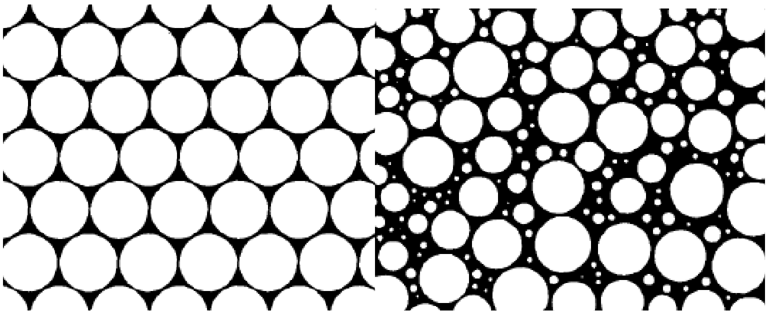


Figure 2. Droplet arrangement in the left is of an homogeneous emulsion whereas the one at the right depicts a non homogeneous emulsion

If the droplets formed were not homogeneous, there occurs a possibility of greater packing density than 74% as the smaller droplets accommodate between the larger ones Both the homogeneous and non homogeneous emulsion are represented in the Figure 2.

1.1.2 Multiple emulsions

Multiple emulsions are the ones in which the dispersed phase particles are themselves the emulsions, these emulsions are prepared from oil and water by re-emulsification of an existing emulsion so as to provide two dispersed phases [4]. A multiple emulsion is the one in which both types of emulsions exist simultaneously. For instance an oil droplet may be suspended in an aqueous phase which in turn encloses a water droplet, thus giving rise to the formulation of w/o/w emulsion. The morphological features can be generalized as follows:

-each dispersed globule in w/o/w (or) o/w/o emulsions forms a vesicular structure with single or multiple aqueous (or oil) compartments separated from the aqueous (or oil) suspending fluid by a layer of oil (or aqueous) phase compartments [5];

-emulsions are formulated for all major routes of administration and there are number of dermatological, oral and parenteral preparations. These types of emulsions have the potential of delivering drug to the specified targets in the body without causing any deleterious effect of the drug to other organs and at the same time these types of emulsions are also used to prolong the release of the drugs that have very short biological half life.

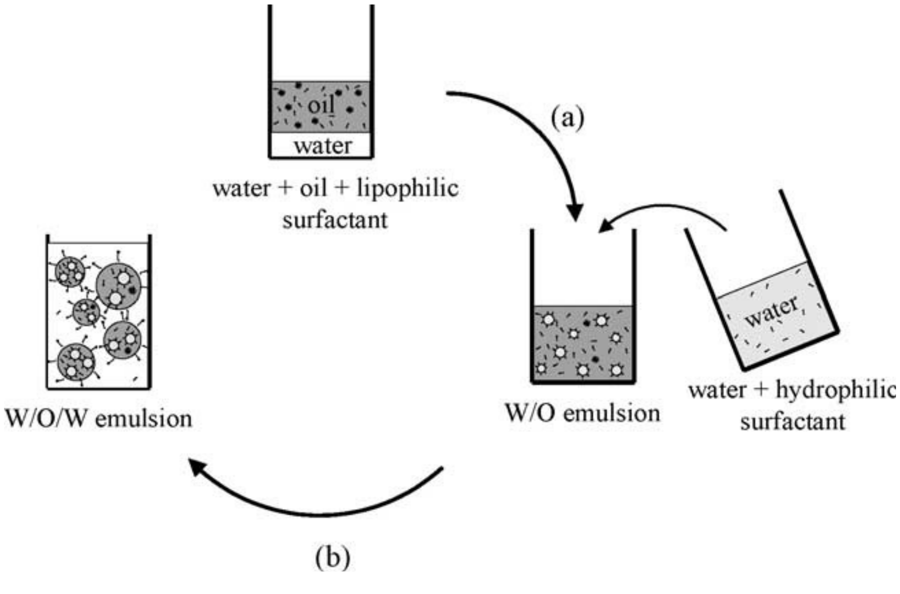


Figure 3. Preparation of w/o/w emulsion by two step process; (a) high shear emulsification method with lipophilic surfactant; (b) low shear emulsification with hydrophilic surfactant

Multiple emulsions are generally prepared by two step procedure, for instance a pre formed w/o emulsion is added slowly, with stirring, to an aqueous solution containing a hydrophilic emulsifying agent (Figure 3). In order to obtain good yields of w/o/w emulsions, a high concentration of the lipophilic emulsifying agent in the oil phase is needed during the preparation of the w/o emulsion and a low concentration of the hydrophilic emulsifier in the aqueous phase during the formation of w/o/w emulsion is required.

Multiple emulsions deserve leading importance in the drug delivery. Vladisavljevi´c et al. [6] formulated w/o/w multiple emulsion with an anticancer drug namely epirubicin and suggested that the clinical trials illustrated multiple drug emulsion when administered directly in to liver via the hepatic artery was effective in contracting the liver cancer texture.

Concerning the stabilization of the droplets, surfactants play a vital role in reducing the interfacial tension thereby increasing the stability [7]. Surfactants are imparted to improve emulsion stability by decreasing interfacial free energy and providing a mechanical barrier to droplet coalescence. The collision of dispersed phase droplets with themselves or with the walls of the container can lead to thinning and rupture of the surfactant interfacial film. Consequently, droplet coalescence and eventual phase separation will occur. This effect can be overcome by the presence of excess surfactant in the bulk, which refills the interface when the film ruptures or thins. Therefore, excess surfactant is usually present in emulsion systems and may be in the form of monomers, micelles, and liquid crystals [8]. The role of surfactant in emulsion is in detailed discussed in the section 1.2.2.

The stability of an emulsion is firstly assessed by the measurement of the size of the inner phase particles. It follows that measuring the size and size distribution of droplets in an emulsion is an important characterization tool, as the size of the droplets influences the viscosity and stability of the emulsion, important characteristics for its processing and functionality [9]. The Stokes equation relates the stability by giving the expression for sedimentation and creaming which is

$$\frac{dh}{dt} = \frac{2(\rho_1 - \rho_2)\, r^2 g}{9\eta} \qquad \textbf{\textit{Equation}}(2)$$

where *dh/dt* is the change in height with time, i.e., the velocity, ρ_1 and ρ_2 are the densities of the phases 1 and 2. This relation can also be explained by the Einstein – Stokes equation which is given by

$$d(H) = \frac{kT}{3\pi\eta D} \qquad \textbf{\textit{Equation}}(3)$$

where *d (H)* is the hydrodynamic diameter, *D* is the diffusion coefficient, *k* is the Boltzmann's constant, *T* is the absolute temperature and η is the viscosity.

1.1.3 Size of the emulsion droplets

The size distribution of the droplets is a very critical factor in the formulation of an emulsion. The droplet size distribution of an emulsion governs emulsion properties such as long-term stability, texture and optical appearance. Consequently, means to control the droplet size during emulsification are of interest when well-defined emulsion properties are needed.

The desired characteristics of an emulsion depend on emulsification and preparation parameters such as type of homogenizer, time of homogenization, number of cycles etc. In mechanical emulsification techniques, the extensional and shear flows employed to break up the inter phase between the immiscible liquids are very inhomogeneous so that, in general, the offspring droplets present a very broad size distribution. Hence, an accurate measurement of droplet size distribution, along with other characteristics such as pH and viscosity, is of paramount importance to analyze the bulk properties and stability of these complex fluids. For example, pharmaceutical emulsions for parenteral nutrition ideally require a fine dispersion of submicron droplets with a narrow dispersion, or low degree of polydispersity, to assure a long shelf-life stability (e.g., to potentially embolic globules). These are usually classed in to "mini-emulsions" corresponding to a typical mean droplet size $d < 1$ µm. In contrast to "micro-emulsions" $d < 0.1\ \mu$m, "mini-emulsions" and "macro-emulsions" ($d > 1\ \mu$m) are thermodynamically unstable. Micro emulsions possess greater stability than the macro emulsions due to the fact that the droplets in the macro emulsions are larger than the other types of emulsions. These larger droplets are a sign of instability as the smaller droplets collide with each other forming larger droplets. Figure 4 represents the size ranges of the dispersed phase droplets in an emulsion, categorization of emulsions based up on the size.

When the emulsion is viewed perpendicular to the incident light. Here the author has demarcated the size of the emulsion droplets as macro emulsions for the droplets ranging from 3 µm to 100 µm and the droplets from 0.1 µm to 3 µm as fine macro emulsions and the droplets between 0.01 µm and 0.1 µm as micro emulsions, the size of the micellar solutions ranges between 0.001 µm to 0.01 µm. According to Somasundaran [10], macro emulsions can be defined as a heterogeneous system consisting of at least one immiscible liquid dispersed in another in the form of droplets whose diameters generally exceed the size of about 0.1 µm, these systems posses a minimal stability which must be accentuated by additives such as emulsifiers, solids etc. These emulsions are turbid and in nature further he defines micro emulsions as a heterogeneous system consisting of at least one immiscible liquid dispersed in another. He adds further that the dividing line between the size of a swollen micelle (0.01 µm – 0.14 µm) and a fine emulsion (0.1 µm – 0.6 µm).

In Pharmaceutical industry, emulsions reserve a prime importance. The size of the emulsion droplets is the factor which also determines the category, the usage of the emulsion. Emulsions with reduced size droplets find there application as intravenous supplements where the emulsions can be administered by the intravenous route, (e.g) *Lipofundina.* ^{TM} The use of emulsions as blood substitute was suggested by the extraordinary solubility of oxygen and carbon dioxide in certain perfluoro chemicals. The use of emulsions as blood substitute was suggested by the extraordinary solubility of oxygen and carbon dioxide in certain per fluorochemicals. This fluorocarbon emulsion could be used for transfusion in such emergencies as a shortage of whole blood [11]. This is especially due to excellent O_2/CO_2 solubility, certain perflurocarbon compounds have found to have even higher solubility for oxygen as compared to the natural blood erythrocytes, e.g Fe-80 which has 10% and posses very higher solubility of oxygen. Perftec™ is one of the perflurocarbon emulsions that allows for efficient intra-vascular transport of oxygen to all cells throughout the body.

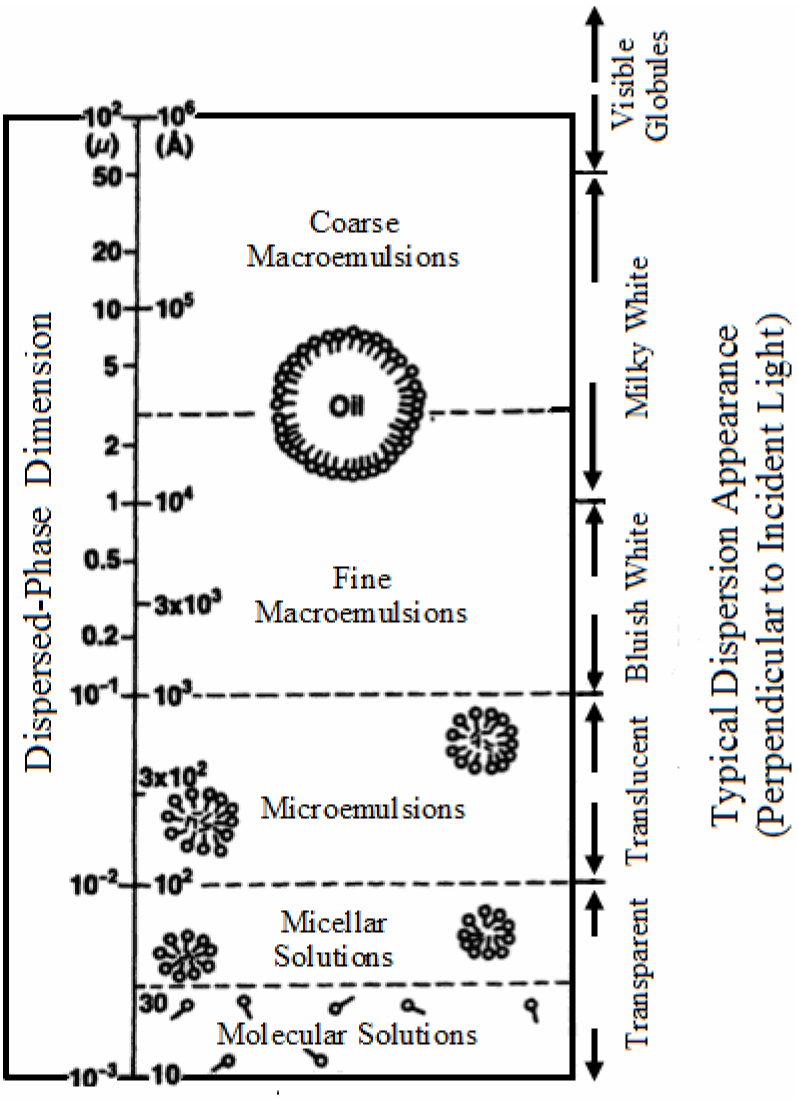


Figure 4. Size ranges of the disperse phase in the emulsions [3]

An attempt has been made to study the stability kinetics from an unstable emulsion. This approach explains the kinetics of the emulsification so macro emulsions are given prime importance in this study.

1.1.4 Methods of emulsification

The methods of emulsification can be divided in to two types they are Low energy emulsification and High energy emulsification.

1.1.4.1 Low energy emulsification

The emulsification can be achieved by different low energy emulsification methods such as a) stepwise addition of oil to a water surfactant mixture; b) stepwise addition of water to a solution of the surfactant in oil; and c) mixing all the components in the final composition, pre-equilibrating the samples prior to emulsification [12]. The commonly used equipments are mixers and vessels with high speed stirrers.

When we consider the equation $W_{min} = \gamma_1 \Delta$, the minimum work spent here would be very less.

Phase inversion helps in the formation of emulsions which also contributes to less expense of energy; usually emulsions are prepared at a raised temperature and not at the room temperature as the raised temperature cause decrease in HLB values and causes inversion. The phenomenon called “phase inversion temperature” reserves a premier importance in this case.

Phase inversion makes it possible for us to change an o/w emulsion into a w/o emulsion or vice versa. The type of emulsion depends, for example, on the order in which the phases were added, the sort of surfactant used, the ratio of phases, the temperature, and the presence of electrolyte or other additives. If water was added to a non polar surfactant solution, a w/o emulsion usually results, whilst addition of oil to an aqueous solution of surfactant yields an o/w emulsion. Temperature-related change in the type of emulsion is caused by the change in surfactant hydrophobicity with temperature.

Addition of an electrolyte can also change the hydrophobicity of the interfacial film and thus cause phase inversion. Strong electrolytes lower the electrochemical potential of the particles, and the interactions between surfactant ions and counter ions are amplified. This can reduce the stability of o/w emulsions (salting out). The inversion of an o/w emulsion stabilized by an interfacial film of sodium cetyl sulfate and cholesterol is depicted in Figure 5. Addition of strong electrolytes, that is, polyvalent cations (in the case represented in Figure 5, Ba^{2+} or Ca^{2+}), neutralizes the charge on the droplets. Small quantities of water are trapped inside the aggregating oil droplets. The molecules in the interfacial film align themselves such that irregularly shaped water droplets are formed, which are stabilized by a rigid, uncharged film, and dispersed in the oil. The coalescence of the oil droplets into a continuous phase completes the process of inversion.

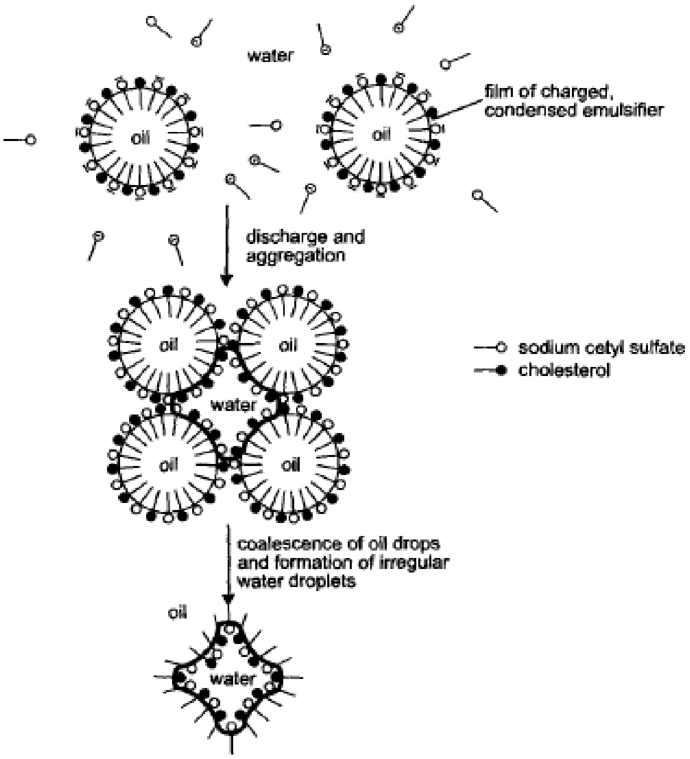


Figure 5. Inversion of an o/w emulsion stabilized by Sodium cetyl sulfate and cholesterol

1.1.4.2 Phase inversion temperature (PIT)

The temperature at which the inversion takes place is called the phase inversion temperature. When the temperature increases the coalescence rate increases proportionately and decreases the stability. This raise in temperature causes thus the hydrophilic groups to suffer the loss of hydrophilicity and cause them less hydrophilic, thus the hydrophilic-lipophilic balance value decreases and this causes the inversion, if an emulsion is o/w type at a temperature which is low, by increasing the temperature w/o can be obtained as inversion occurs. At phase inversion temperature the hydrophilic and lipophilic properties of the surfactant are balanced, moreover the interfacial tension of the surfactant reaches a minimum value in the region of the PIT, at this point it is optimum to emulsify a mixture near the PIT as there forms very small droplets with little energy expenditure.

1.1.4.3 Emulsion Inversion Point

The emulsion inversion point, EIP is the point at which one type of emulsion turns in to another by the addition of the dispersed phase volume for instance a w/o emulsion turns into an o/w emulsion when water is added. It is given in units of cm^3 water added to 1 cm^3 oil. This assumes that the emulsion consists of water, oil, and emulsifier. The EIP value declines with increasing HLB value of the emulsifier; it reaches a minimum that corresponds to the optimum stability of an o/w emulsion. The EIP method is a fast way of finding out the physical stability of emulsions. The most stable o/w emulsions are often manufactured starting from w/o emulsions that are then inverted. P. Fernandez et al. [13] experimented on emulsification by emulsion phase inversion method and suggested that much smaller and finely dispersed oil droplets can be achieved by this method than mechanical emulsification alone.

From the above description phase inversion in emulsions can be one the following types:

-transitional inversion method mainly caused by changing factors which affect the HLB of the system, e.g. temperature and /or electrolyte concentration. This method can also be induced by changing the HLB number of the surfactant at constant temperature using surfactant mixtures; [14]

-catastrophic inversion is the one mainly caused by increasing the volume fraction of the dispersed phase. Shinoda and Saito demonstrated that emulsification at phase inversion temperature followed by cooling led to the formation of stable o/w emulsions of small size. Emulsification at temperature higher than phase inversion temperature o/w emulsions resulted in very stable emulsions on subsequent cooling [14].

1.1.4.4 High energy emulsification

In this type of emulsification process (Figure 6) agitation of oil phase with the aqueous phase with the added surfactant is done to produce the emulsion droplets.

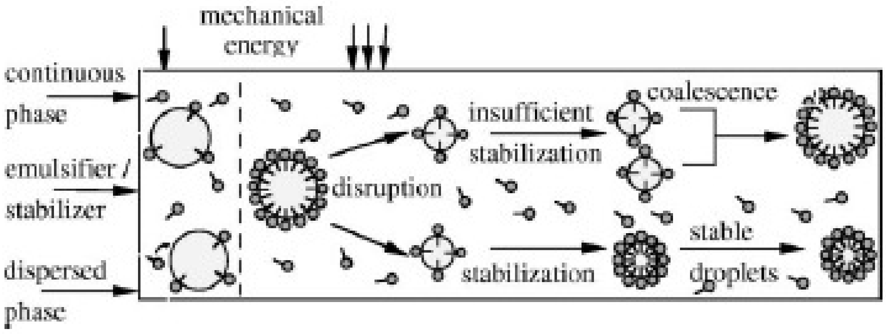


Figure 6. Representation of the Process of mechanical emulsification

This involves supply of high energy. Droplet formation occurs on both the phases when agitated and the continuous phase result since its droplets are unstable. The agitation of water and oil together cause both oil droplets in water and water droplets in oil but the ultimate product is oil in water emulsion as the water droplets coalesce with one another much faster than the oil droplets. The coalescence of sufficiently large number of water droplets leads to the formation of the continuous phase; it surrounds the dispersed phase as shown in the Figure 7. The continuous phase formation occurs more rapidly which usually takes few seconds and is not pertinent to the stability of the emulsion. The decisive factor in the formation of an emulsion is mechanical agitation, stirrers and extrusion equipment.

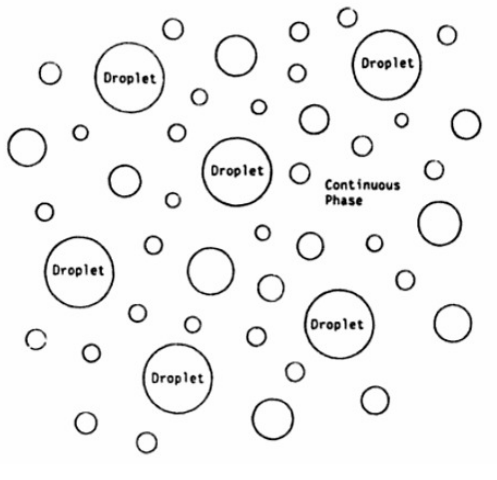


Figure 7. Dispersed phase surrounded by continuous phase

The type of the emulsion whether o/w or w/o is determined by the phase ratio if the numbers are high. [9]. Figure 6 represents the mechanism of the process of mechanical emulsification. Which is self explanatory showing disruption of droplets and further insufficient disruption causes coalescence and stabilization forms stable droplets.

When we consider the equation (1), $W_{min} = \gamma_1 \Delta$, the work input may be higher as the energy input increases and so the minimum energy required may be more to create an interface or the additional interface which is the product of interfacial tension and increase in the area of the interface.

1.2 The study kinetics of emulsification

An emulsion is stable from the kinetic point of view, when the number, droplet size distribution, and arrangement of droplets do not undergo any discernible change over the storage time scale [15]. A considerable amount of mechanical energy is required for the emulsification process. The dispersion of one of the liquids in the form of small droplets in the continuous phase may lead to emulsification. The emulsification process can be expressed as follows, based on the local capillary number

$$C_a = \frac{\eta_c \, \ddot{v} \, R}{\sigma} \qquad \textbf{Equation}(4)$$

Where η_c is viscosity of the continuous phase, $\ddot{v}$ is shear rate, σ/R is the interfacial stress (σ being interfacial tension and R is the radius of the dispersed phase droplets). The capillary (C_a) and Reynolds numbers decrease due to the length scale reduction and the increase in viscosity respectively. The dispersed droplets are stretched when capillary number is much higher than the critical value, $C_{a\,\mathrm{crit}}$. The local stress overrules the interfacial stress. These stresses are usually generated by the turbulent flow or the laminar flow. These stresses were generated at the beginning of the process and leads to the formation of threads, which are large liquid threads. The second step is the breakup of threads and drops which usually occurs when the capillary number (C_a) is higher than the critical value ($C_{a\,\mathrm{crit}}$).

The capillary critical value depends on the ratio between the dispersed phase and on the continuous phase viscosities. Then the last step is the coalescence of the disperse droplets when the capillary number is much lower than the critical capillary value [15].

Intense agitation causes disruption of droplets and their coalescence. There are many variables which govern the evolution of the emulsification process and many variables are related to the processing conditions. There can be numerous process parameters applied, among which the mixing intensity, the order of incorporation of the ingredients and the temperature are the most common. The surface produced in the process of emulsion formation if probably not fully covered by the surfactant then the emulsification conditions are not optimal because the surface tension in the mixture is not less. The interfacial tension of the system evolves with time, with a time scale of approximately 100 s. As a consequence, short residence times in the mixing head may present severe drawbacks. A way to avoid this is to increase the residence time in the mixer, by reducing the flow rate, increasing the active volume [16]. The different processing conditions include the following the:

-type and geometry of the equipment used;

-residence time;

-agitation speed;

-surfactant concentration;

-oil volume;

-rheological properties of the dispersed and the continuous phase involved.

The processing variables influence the many structural parameters yielding dramatic differences in the kinetics and rheology of the emulsions.

The structural parameters and rheological properties govern the control of processing and the stability of the emulsion. In order to tailor-make emulsions with distinct functional properties, the material behaviors under processing and application condition has to be known. Drop deformation behavior and process related flow conditions are to be known. There exists impact of process design and processing parameters on the micro-structure rheology relationships. The final product micro-structure based quality characteristics have to be known [17].

The flow-processing operators for emulsions include laminar and turbulent flow conditions. The laminar flow conditions are applicable for highly viscous system, here mixed shear and elongation flow fields are most frequent. The droplets are deformed, broken up dispersing and possibly shape-fixed micro structuring encapsulation. Drop size and drop distribution interfacial properties and structuring elements in the continuous phase may contribute to the formation of a complex network structure in the emulsion systems. This structure determines the material behavior of the final product under various possible application conditions and is closely related to product quality characterization [17].

1.2.1 Droplet breakage mechanism

Deformation and breakup behaviors of the droplets strongly depend on the rheology of disperse and continuous fluid phases as well as from the interface properties. The ratio of external stress over the Laplace pressure establishes the deformation of a droplet. This can be represented in a dimensionless capillary number (Ω) can given as the following

$\Omega = \frac{\eta_c \gamma r}{\sigma}$, which is equation 4

where η_c (Pa s) is the shear viscosity of the continuous phase and γ (s^{-1}) is the shear rate. The droplet deformation can be given in several means. The droplet deformation increases with increasing capillary number (Ω).

While $\Omega < \Omega_{cr}$, the initial spherical droplet is deformed in to a stable ellipsoid, Ω_{cr} is the critical capillary number. If the capillary number is greater than the critical capillary number, $\Omega > \Omega_{cr}$, a stable droplet shape is not formed; at this point the droplet happens to stretch continuously until it breaks. The external stress dominates the interfacial stress and these stresses yielded at the beginning of the process leads to formation of threads which are large liquid threads that break up. This breakage of droplets occurs when $\Omega >> \Omega_{cr}$ that is the capillary number is higher than the critical capillary number.

Droplet breakage occurs by one of the following four mechanisms:

-necking;

-tip streaming;

-end-pinching;

-capillary instabilities.

1.2.1.1 Necking

Droplets form a globular ends and a neck is developed between them. The thinning of the neck continues until there approaches a breakup. This produces smaller droplets which perfectly positions between two large droplets which generates from the bulbous ends. This necking (Figure 8) occurs mainly when the flow is a sustained flow where Ω is relatively close to Ω_{er}.

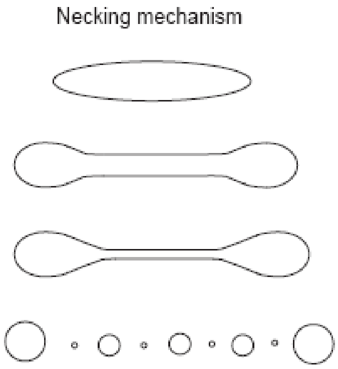


Figure 8. Representation of necking mechanism [11]

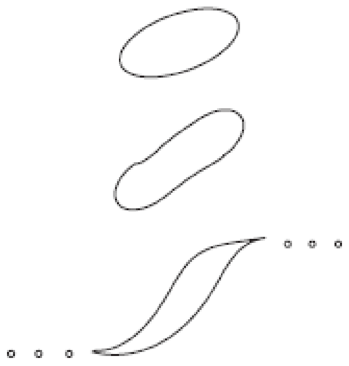


Figure 9. Representation of tip streaming mechanism [11]

1.2.1.2 Tip streaming

Small droplets are produced. This occurs by breaking off from the tips of the moderately extended, pointed droplets (Figure 9). Tip streaming occurs mainly due to the presence of surfactants.

1.2.1.3 End pinching

This is a typical mechanism where the moderately extended droplets are influenced by the surface tension forces and this occurs when the shear rate is very low. End pinching (Figure 10) commences when the droplet gets deformed beyond its maximum steady shape and then after flow stoppage, the droplet gets relaxed back towards its spherical shape but break in to fragments structuring a number of smaller droplets

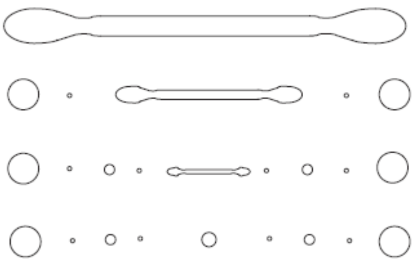


Figure 10. Representation of end pinching mechanism [11]

1.2.1.4 Capillary instabilities

Capillary instabilities occur when a droplet is subjected in to sudden stress much greater than the critical stress for breakup ($\Omega >> \Omega_{cr}$), the droplet gets stretched affined and becomes highly extended thread. The extended droplet is very unstable to slight disturbances and will eventually disintegrate into number of large droplets with satellite droplets in between. A schematic representation is shown in the Figure 11.

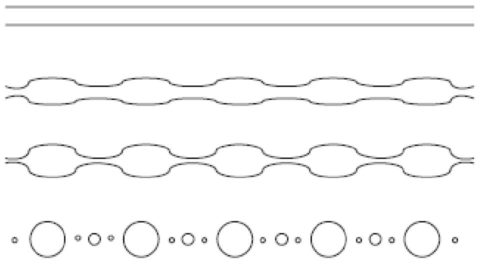


Figure 11.Capillary instability mechanism

1.2.1.5 Droplet breakup in concentrated emulsions

The break up discussed as yet considers the breakage of a single droplet without the presence of a surfactant. In actual emulsification process the discussed breakage mechanism may not be rational. When we consider the emulsification process, the surfactants are present; the droplet may be subjected to continuously changing hydrodynamic conditions and the droplet breakage occurs in the presence of large population of droplets of different sizes. Several authors present an experimental study on conditions for droplet break up in concentrated emulsions under simple shear flow and put forward that critical capillary number for breakup decreased by more than an order of magnitude for the most concentrated emulsions and thus assumed that the breakup of droplets in concentrated emulsion is determined by the average emulsion viscosity rather than the continuous phase viscosity, which can be expressed as $\Omega = \frac{\eta_c \gamma r}{\sigma}$, which is previously explained in Equation 4.

Here η_e [Pas] is the emulsion viscosity, γ (s^{-1}) is the shear rate, σ is the interfacial tension. [11].

1.2.1.6 Over Processing

Over processing is one of the phenomena that occur during the high energy emulsification. Some of the reasons are:

-the poor adsorption of the surface active agent to the interface

-low residence time of the emulsion in the emulsification zone;

-high rate of coalescence frequency;

-extreme amount of energy density.

Despite the fact of increase in the energy input during emulsification, the obtained emulsions would obtain bigger droplets than the expected smaller droplets. This is the phenomenon of over processing [18].

Several authors studied that the above development in high-pressure emulsification.

There are two ways to avoid recoalescence immediately after the droplet disruption:

(1) Fast stabilization of new interfaces by sufficient surfactant molecules;

(2) Stabilization by retardation of the liquid drainage between two colliding droplets with the increase of continuous phase viscosity or by increasing the collision time by a turbulence flow field after the dispersion zone.

1.2.2 Role of the Surfactant

1.2.2.1 Surfactant

Surfactant is a contraction of the term surface active agent which at its low concentration in a system has the property of adsorbing on to the surfaces or interfaces of the system and of altering to a marked degree the surface or interfacial free energies of those surfaces or interfaces. An interface is a boundary between any two immiscible phases. (Figure 12) represents the interfacial layer between the two phases [5]

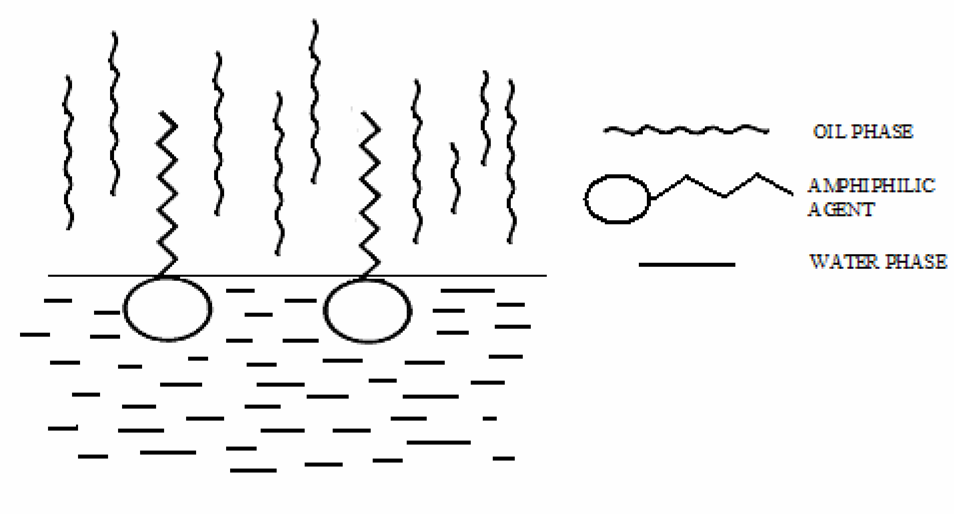


Figure 12. Interfacial layer between the oil and the water phase

A surfactant (Figure 13) is a substance that at low concentrations adsorbs at some or all of the interfaces in the system and significantly changes the amount of work required to expand those interfaces

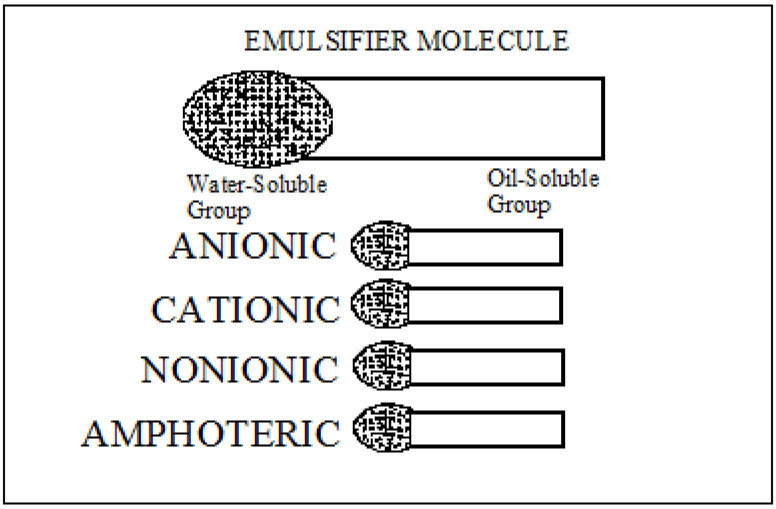


Figure 13. Representation of types of emulsifier molecule

Surfactants act to reduce the interfacial free energy Figure 14[7].

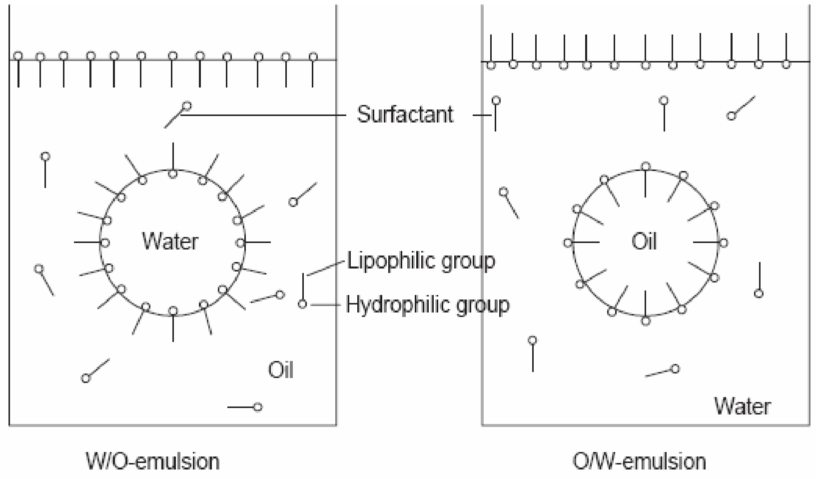


Figure 14. Representation of o/w and w/o emulsions containing hydrophyllic and lipophilic group of the surfactant

The physical, chemical and electrical properties of matter confined to phase boundaries are often profoundly different from those of the same matter in bulk. A fraction of total mass is often localized at phase boundaries but is so small that the contribution of these abnormal properties to the general properties and behavior of the system is negligible but in one circumstance is when the phase boundary, area is so relative to the volume of the system that a significant fraction of the total mass of the system is present at boundaries as in *emulsions,* foams, dispersions of solids and here a vital role is played by surfactants. The role of surfactant molecules is a requisite typically to improve both emulsification process and emulsion stability. By adsorbing at the interface they reduce the interfacial tension and causes easy breaking up of the droplets. They play vital roles in deformation and break up of droplets [15]. Affinity of surfactants to the interfacial region facilitates the formation of rigid film of the emulsifier at the interface that acts as a mechanical barrier to droplet adhesion and coalescence (Figure 14). The supply of more emulsifier to the interface must be assured so that the rate of diffusion from the bulk solution to the interface must be adequate [3]. Coalescence is reduced by increasing the surface viscosity and the movements of the interface are greatly reduced. The emulsion stability increases as the surface viscosity and yield value of the film increase. They also hinder the deformation of the interface. If the surfactant distributions remain not so uniform at the interface, instabilities linked to interfacial tension gradients may develop. The adsorption time of the surfactant is compared with the deformation time of the droplets and residence time in the mixing head. The type as well as the amount of a surfactant immediate effect on the type and stability of the resulting emulsion. The molecular structure of surfactants consists of:

a) hydrophillic group: the group which has a strong attraction for solvents;

b) lipophillic group (or) hydrophobic group: the group which has a very little attraction for solvents;

c) amphipathic structure: the group which posses both the hydrophilic and lipophyllic group.

The hydrophobic part is a long hydrocarbon chain that could be branched also. The hydrophilic part could be anionic or cationic or simply a polar group as represented in Figure 13. The surfactant tends to be soluble in one of the liquid phases. Thus it concentrates at the interface. Table 1 represents the surfactants types. The types of surfactants which have a stabilizing effect on the interfacial film between oil and water phases may be encountered as:

-ionic surfactants;

-non-ionic surfactants;

-amphoteric surfactants.

Ionic surfactants

1. Anionic surfactants are the type in which the water soluble group is negative, the head group is negatively charged.
2. Cationic surfactants are the type in which the water soluble group is positive, the head group is positively charged.

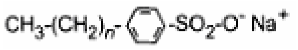
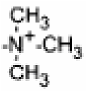
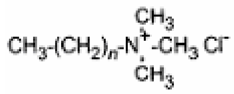
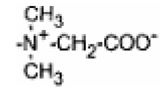
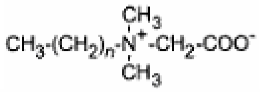
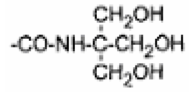
Nonionic surfactants:

The surfactants do not posses any charged group.

Zwitterionic (amphoteric) surfactants

A surfactant possessing two oppositively charged groups.

Table 1. Surfactants grouped by type

Type	Hydrophilic substituent	Example	Name
anionic	$-COO^-$	$CH_3-(CH_2)_n-COO^- Na^+$	soaps, salts of fatty acids
	$-O-SO_2-O^-$	$CH_3-(CH_2)_n-O-SO_2-O^- Na^+$	sulfates of fatty alcohols
	$-SO_2-O^-$	$CH_3-(CH_2)_n-SO_2-O^- Na^+$	alkyl sulfonates
			alkyl aryl sulfonates
	 $-CO-\underset{R}{N}-CH_2-CH_2-SO_2-O^-$	 $CH_3-(CH_2)_m-CO-\underset{R}{N}-CH_2-CH_2-SO_2-O^- Na^+$	fatty acylated aminoethyl sulfonates
cationic	 $-\overset{CH_3}{\underset{CH_3}{N^+}}-CH_3$	 $CH_3-(CH_2)_n-\overset{CH_3}{\underset{CH_3}{N^+}}-CH_3\ Cl^-$	Alkyl trimethyl ammonium chloride
zwitterionic	 $-\overset{CH_3}{\underset{CH_3}{N^+}}-CH_2-COO^-$	 $CH_3-(CH_2)_n-\overset{CH_3}{\underset{CH_3}{N^+}}-CH_2-COO^-$	*N*-alkylbetaine
nonionic	$-O-(C_2H_4-O)_nH$	$CH_3-(CH_2)_m-O-(C_2H_4-O)_nH$	Polyethylene oxide alkyl ether
			Polyethylene oxide alkyl aryl ether
H-bond active	 $-CO-NH-\overset{CH_2OH}{\underset{CH_2OH}{C}}-CH_2OH$	 $CH_3-(CH_2)_n-CO-NH-\overset{CH_2OH}{\underset{CH_2OH}{C}}-CH_2OH$	fatty acyl derivatives of trimethylolaminomethane

When stable oil in water emulsion is produced using a cationic surfactant the droplets will develop a positive charge on their surfaces. Thus the type of charge imparted to the droplet can be easily deduced from the type of the surfactant used. The factors responsible for determining the type of the emulsion are discovered as follows:

> -the dispersed phase may be either 1) molecularly soluble (or) 2) colloidally dispersible in water. If the dispersed phase is colloid ally dispersible it should be either hydrophobic or hydrophilic. If an oil soluble hydrophilic colloid is dissolved in oil, it is impossible to attain water soluble hydrophobic colloid is dissolved in oil in water dispersion. A hydrophilic surfactant will favor the formation of oil in water emulsion, whereas a hydrophobic surfactant will favor the formation of water in oil emulsion. The surfactant is more soluble in the external phase however any surfactant must have the ability to come in contact with the oil. The surfactant molecules adsorb at the oil in

water interface, quoted previously reduces the interfacial tension, yielding a decrease in the amount of mechanical energy required to deform and disrupt the droplets and favors the emulsification. They also help to prevent recoalescence caused by the formation of interfacial tension gradients during emulsification [15].

The stability of an emulsion is influenced by the charge at the interface and by packing of the surfactant molecules. As a matter of fact, extremely low interfacial tension (Figure 15) may lead to instability.

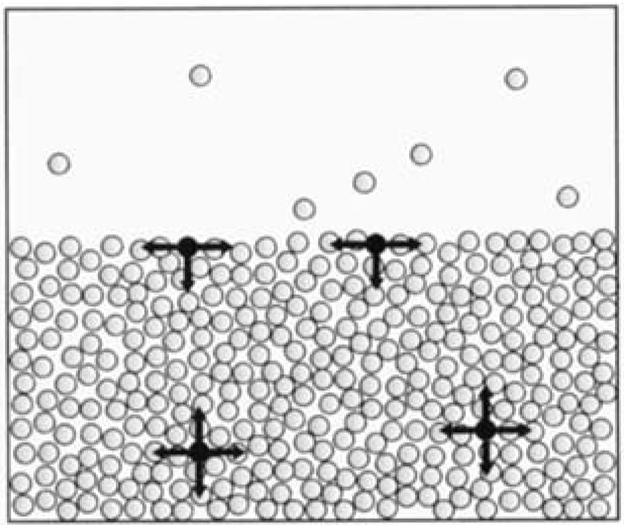


Figure 15. Representation of surface tension of a particle in an emulsion

The selection of the type of the surfactant deserves a prime importance in order to prepare emulsion of a particular type.

Hydrophilic-lipophilic balance (HLB):

The hydrophile- lipophile balance is a way of predicting the emulsion type from surfactant molecular composition, surfactants with low HLB ($\leq$ 6) provide stable w/o emulsions whereas surfactants with high HLB value for instance ($\geq$ 8) stabilize o/w emulsions (Table 2).

Table 2. HLB estimation of surfactants based on water dispersability

HLB range	Water dispersibility
1 – 4	Not dispersible
3 – 6	Poorly dispersible
6 – 8	Milky dispersion only after vigorous agitation
8 – 10	Stable milky dispersion
10 – 13	Translucent to clear dispersion
> 13	Clear solution

One of the important effects of an emulsifier is its interfacial adsorption rate that determines the stabilization of newly broken up droplets against the coalescence.

1.2.3 Energy requirement

Energy is one of the important components in the production of the emulsion apart from oil, water and surfactant. In the process of emulsification energy is essential to expand the interface which can be expressed as $\Delta A\gamma$, where ΔA is the increase in interfacial area. For instance when we consider the bulk oil with area A_1 produces a large number of droplets with area A_2 then $A_2 >> A_1$. Here γ is the interfacial tension [12].

As emulsion formation is non-spontaneous so the energy plays a vital role in the production of the droplets, formation of larger droplets is fairly easier using mechanical agitation than smaller droplets. Production of smaller droplets requires high amount of energy than larger droplets. This can be understood considering the Laplace pressure. The Laplace pressure is given as the Pressure difference between inside and outside of the droplet.

$$P = \gamma\left(\frac{1}{R_1} + \frac{1}{R_2}\right)$$ ***Equation** (5)*

where R_1, R_2 are the principal radii of curvature of the drop. In case of a spherical drop $R_1 = R_2 = R$ and$P = \frac{2\gamma}{R}$, spherical droplet has only one radius of curvature R_a, for prolate ellipsoid there are two radii of curvature R_{b1} and R_{b2}

The essential factor in the efficiency of mechanical agitation to produce small droplets is the ratio between the Laplace pressure and stress from shear gradient [9].

The breaking of drops in to droplets occurs when the Webner number is in excess of the value 1. Webner number (W_e) of a droplet can be defined as the ratio of kinetic energy due to fluctuations to the energy due to interfacial tension [1].

Webner number (W_e) can be expressed as

$$W_e = \eta Gr/\gamma \qquad \textbf{\textit{Equation}}\ \textit{(6)}$$

where η is the viscosity of the continuous phase, G is the velocity gradient (intensity of mechanical agitation); r is the radius of the droplet and $\gamma$is the interfacial tension. In order to attain the break up of drops in to smaller droplets, the drops must be strongly deformed; this deformation increases Laplace pressure (P). The stress needed to deform the drop is higher for a smaller drop as the stress is generally transmitted by the surrounding liquid via agitation; these higher stresses need more vigorous agitation, hence more energy is needed for smaller drops.

1.2.3.1 Process equipment

Emulsions can be produced using the following systems:

-rotor-stator systems;

-high-pressure systems;

-membrane systems;

-ultrasonic systems

Rotor-stator systems

Discontinuous and continuous production includes this system. Semi-continuous production requires agitators of different geometry to serve this purpose gear-rim disperstators, gear-rim dispersion machines and intensive mixers are used. This rotor stator assembly (Figure 18) consists of rotor housed concentrically inside the stator with either vertical or slant slots [18]. As the rotor rotates, it generates a low pressure to draw the liquid in and out of the assembly, resulting in circulation and emulsification. The drops of the disperse phase are broken up to a large extent by forces of inertia and shearing in turbulent flow. In colloid mills also shear stresses in laminar flow can lead to droplet breakup. Mean droplet diameters ranging above 1μm could be achieved with these systems. On the other hand with continuously operated rotor-stator systems there exists increased product output, as the product stress is very high in most cases. The scheme of the emulsification process is represented in Figure 16.

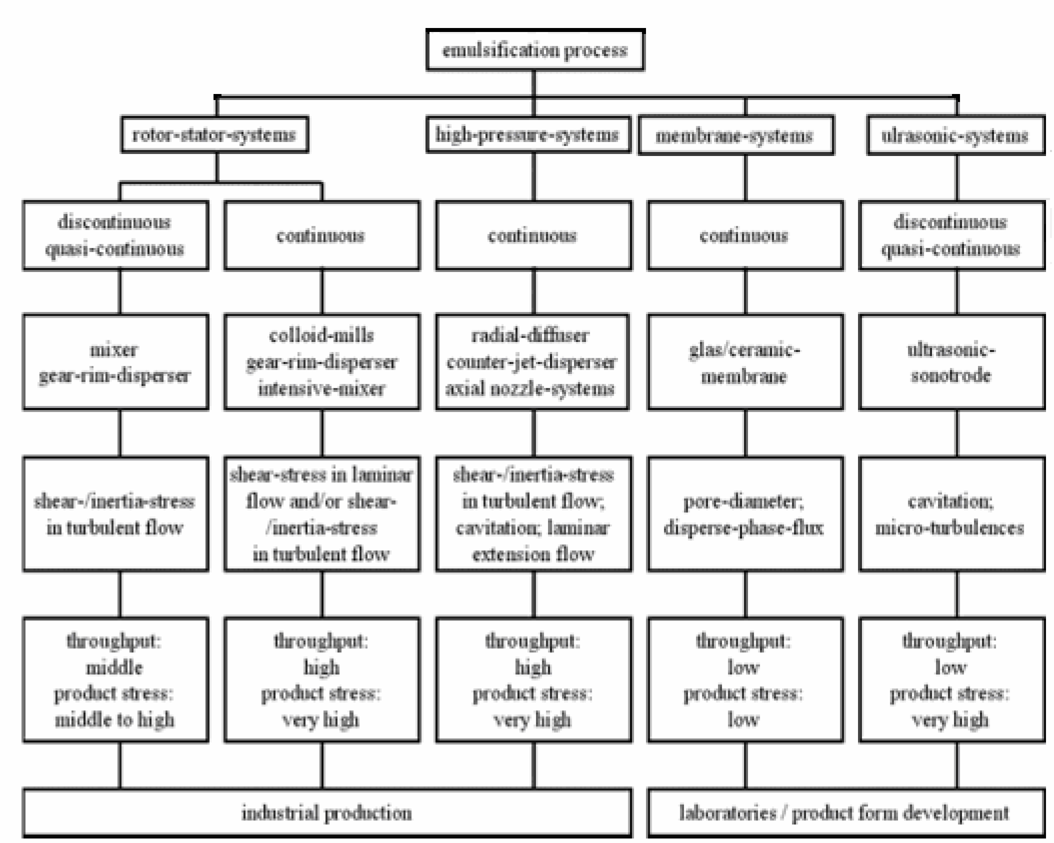


Figure 16. Scheme of emulsification process[23].

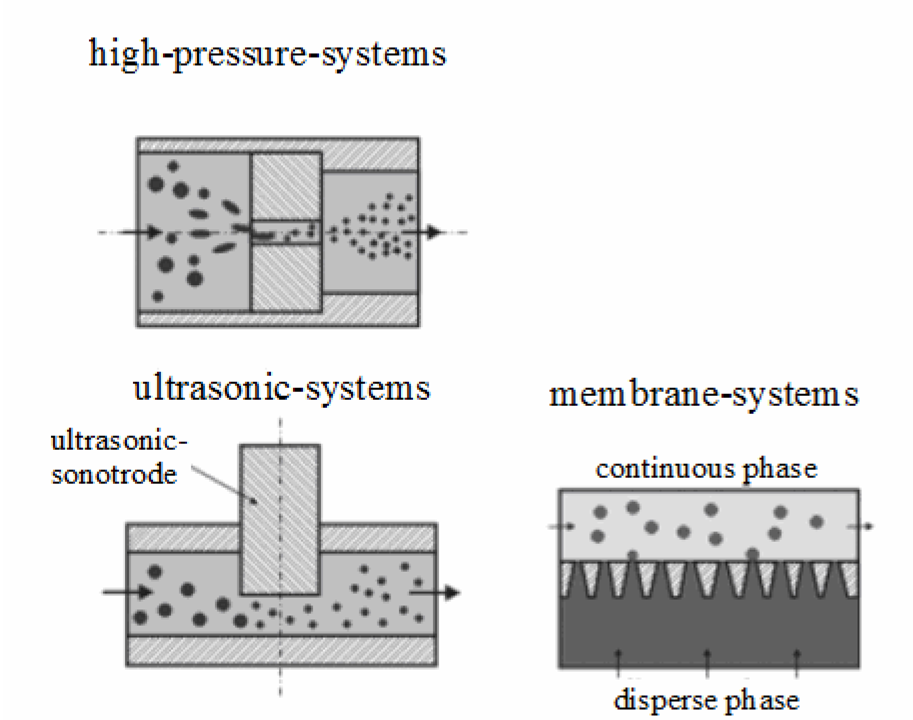


Figure 17. Different emulsifying systems[23].

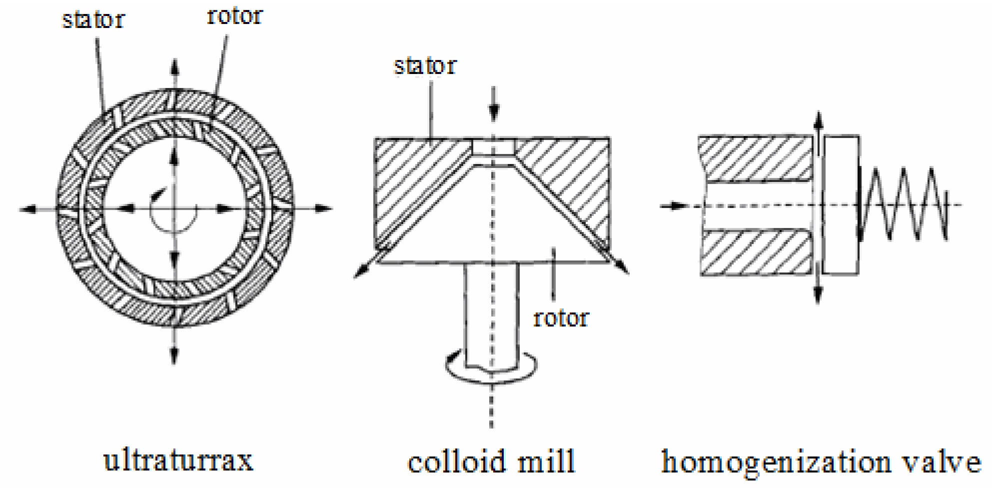


Figure 18. Examples of Emulsification apparatus[23].

High-pressure systems

In high pressure systems (Figure 17), the main categorization counts on nozzle geometry, design and on the flow guidance. In this system the emulsifying nozzle is critical for the efficiency of disruption. Some of the high pressure systems are radial diffusers, counterjet dispergators, axial nozzle aggregates, and these systems can be applied depending on the flow guidance.

Table 3. Comparison of different Emulsification systems [23]

Emulsification system	Rotor-stator system	High-Pressure system	Ultrasonic system	Membrane system
Examples	Mixers, agitators, colloidmills (Silverson, UT)	Radial diffusers, valve homogenizers, Jet diffusers, microfluidizers	Sonotrodes (sonication probes)	Glass/ceramic membranes
Droplet disruption mechanism	Shear stress in laminar flow and/or shear and inertial stress in turbulent flow	Shear and inertial stress in turbulent flow cavitation in laminar flow	Cavitation in micro-turbulent flows	Dispersed phase flux
Through put	Medium to high	High	Low	low
Batch/continuous	Batch(mixer) or continuous colloid mills	continuous	Batch or quasi continuous	continuous
Minimum droplet size (m)	1	0.1	0.1 – 0.2	0.2 – 0.5
Optimal range of viscosity	low to high (20 – 5000 mPas)	low to medium(1 – 200 mPas)	low to medium	low to medium
Application	Lab/Industrial	Lab/Industrial	Lab	Lab
Energy density	low to high	medium to high	medium to high	low to medium
Change of energy input through	Rotation speed, expensive time, gap distance and disk design	Pressure, recirculation(exposure time) and nozzle design	intensity frequency of ultrasonic wave sonication	Pore diameter
Residence time in dispersing zone t	$0.1 < t < 1$s	$0.1 < t < 3$ms	-	-
Required adsorption rate of emulsifier	Low to high	high to very high	Middle to high	Medium to high

This system is always employed in continuous operation. In these systems forces of inertia and shearing in turbulent flow causes the droplet breakup, other mechanisms like cavitations and shear stresses in laminar extension flows contribute to droplet disruption. High pressure systems contributes to mean droplet diameter of about 0.2µm with high product outputs, still the stress on the product is very high due to high pressure gradients and flow rates. Table 3 represents the comparison of different emulsification systems.

Membrane emulsification

Membrane emulsification using micro porous glass or ceramic membranes represents a relatively new continuous emulsification process. Here the dispersed phase is pressed and passed through the pores of a membrane. At the pore outlet the formed drops are entrained by the continuous phase which is flowing along the membrane. Very low droplet diameters of about 0.2 µm with narrow droplet size distributions can be obtained. This system causes very less stress to the products and it can be the suitable system for the stress sensitive products.

Ultrasonic systems

In Ultrasonic systems the emulsification process involves the energy input from so called Ultrasonic sonotrodes. They are the sonicator probes. As the tip of the sonicator probe contacts the liquid, mechanical vibrations and cavitations occur. The main mechanism of droplet break up is by means of cavitation, microturbulences also cause the droplet disruption.

The droplet diameter ranges from 0.4µm. The product stress remains very high here. This system may not be very useful for stress sensitive products.

There exists wide variety of emulsifying devices depending on design and functional capability, the choice of the emulsification apparatus is determined by the following factors:

-emulsion volume;

-viscosity of emulsion and its phases;

-surfactant type and concentration;

-temperature considerations;

-final droplet size distribution.

Selection of the emulsification apparatus according to the suitability of the above mentioned parameters, the operating conditions such as flow rate, pressure, gap thickness, temperature, emulsification time and rotational speed should be optimized.

Also Energy density is a liable parameter that can contribute to compare the efficiency of emulsification of different devices. Energy density (E_v) can be given as the amount of energy E dissipated per unit volume of the emulsion $V_{e.}$ The total power input (P) and the flow rate V_e gives the energy input for the continuous emulsification which can be expressed as:

$$E_v = E / V_{e.} = P / V_e \qquad \textbf{Equation } (7)$$

In mechanical emulsification the viscosity of the dispersed phase influence the droplet disruption but the continuous phase viscosity does not produce any effect provided energy density is constant and coalescence is avoided [19]. The disruption of droplets in rotor stator system was found to be less efficient than in high pressure devices. According to this author, the dispersing zone of rotor stator systems usually has larger volumes. The mean residence time is longer in rotor stator system than in the nozzles of high pressure devices. The operating forces in the rotor-stator device act longer than the minimum time needed for the droplet breakup. The efficiency of droplet disruption in turbulent flow increases in direct proportion to the power density and inverse proportion to the length of residence time in the dispersing zone of the emulsifying device.

Studies on Ultra Sound emulsification have shown that the smaller droplets produced by this method was more efficient than rotor-stator systems [1]. Studies on microfluidization indicate that it is the superior method to other emulsification devices, because the droplet size distribution appeared to be narrower and smaller in microfluidized emulsions than in other systems.

1.2.4 Stability of emulsions

The stability of an emulsion is mainly determined by the properties of the interface. In this study, stability of two-phase emulsion is discussed. A stable emulsion can be easily described practically as an emulsion through observation. The separation does not change with time. To study about the stability of an emulsion, the nature of stability of an emulsion must be compared with the question of instability of an emulsion. The final state of an unstable emulsion is easy to observe. The separation of oil and water layer occurs. The oil layer separates out and exists over the aqueous layer. This is the final state of an unstable emulsion (Figure 19). The detection of earlier phenomena of instability is very significant.

A supreme attention need to be focused on the initial mechanism in many process involved in the destabilization of an emulsion. Emulsions are unstable exhibiting flocculation, coalescence, creaming and degradation. This occurs due to the spontaneous tendency toward a minimal interfacial area between the dispersed medium. Minimizing the interfacial area is mainly achieved by two main mechanisms, first by flocculation of droplets followed by coalescence and then second by Ostwald ripening.

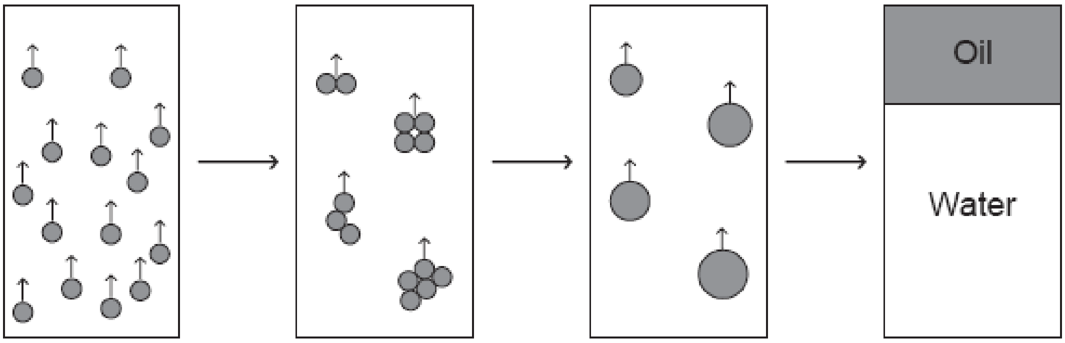


Figure 19. Stages of instability in emulsion showing flocculation followed by coalescence and finally phase seperation

1.2.4.1 Flocculation

Flocculation is the first process in the start of the instability process. Flocculation occurs when two droplets become attached to each other, these droplets are separated by a thin film of the liquid. These droplets form a cluster and form an aggregate. These aggregate still maintain a thin film between them. The next step is Coalescence.

1.2.4.2 Coalescence

Coalescence is often considered as the most important destabilization mechanism causing coarsing of dispersions (Figure 20). Breaking of the emulsion is mainly because of Coalescence. The disruption of the droplets occurs during the coalescence and follows the phase separation and the emulsified system gets destroyed. Therefore, the rate of coalescence of the droplets was chosen as the only quantitative measure for the stability of an emulsion.

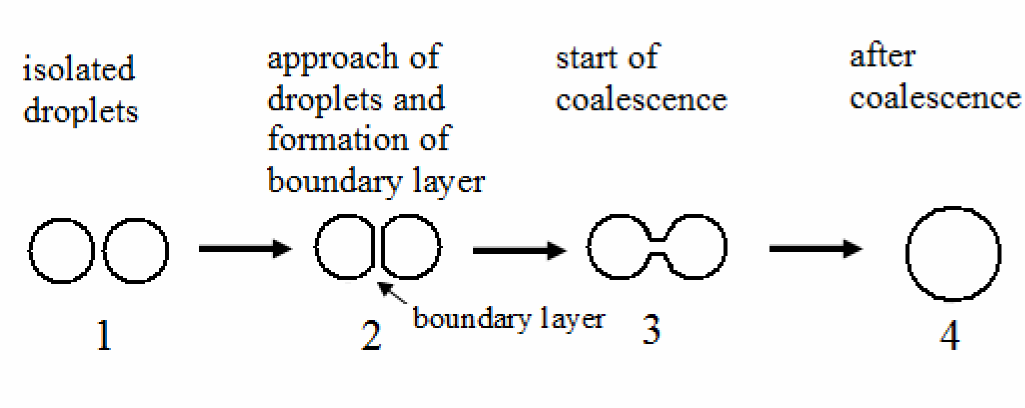


Figure 20. Coalescence in process in less stabilized droplets

Ostwald ripening always found to be the main mechanism causing instabilities. This mainly arises from difference in solubility between droplets with different sizes. Larger the droplets grow at the expense of smaller ones due to molecular diffusion through the continuous phase. Coalescence is usually seen as a wide distribution of droplets but no clusters are seen. These large droplets undergo creaming. Coalescence is the factor causing the creaming or sedimentation. The coalesced droplets cream or sediment much faster than the normal droplets. The rate of this process is given as:

$$V = \frac{2\Delta P r^2 g}{9\eta}$$

***Equation** (8)*

where v is the sedimentation velocity, ΔP is the difference in density between the droplet and the difference in the continuous phase; g is the gravity acceleration and η is the viscosity of the continuous phase. It is clear that droplets that are much larger are faster collected on the top, this process is called creaming. When the droplets accumulate at the bottom it is called sedimentation (Figure 21) [20].

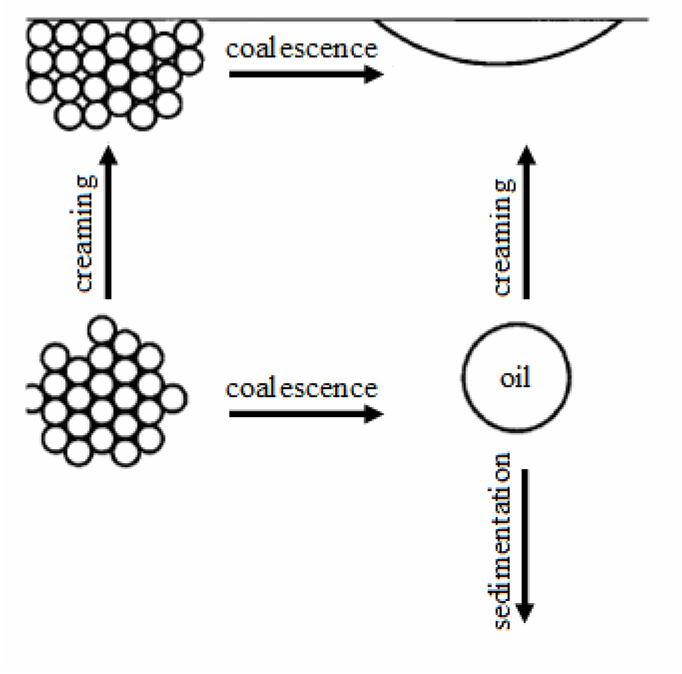


Figure 21. Sedimentation and creaming of o/w emulsion

One property that makes emulsion more special is the deformability of individual droplets, large droplets are easily deformed where as the smaller ones approximately in a size range 1µm in diameter, are assumed to behave more like hard spheres due to their large Laplace pressure. Under low interfacial tension even small droplets can become deformable, resulting in inter-droplet attraction and coalescence-enhanced creaming but when surfactant molecules are present they reduce coalescence by increasing surface viscosity by reducing movements of the interface and hinder the deformation of the interface.

In o/w emulsions, when charged emulsifiers are used, the hydrophilic part of the surfactant may face the water and the electrical charge on the droplet acts as a barrier to prevent coalescence. For ionic surfactants, the sign of the charge on the droplet is the same as that on the surfactant, while non ionic emulsifiers are not charged so the dispersed phase would be charged either by adsorption of ions from the aqueous phase or by the motion and friction of the droplets in dispersion medium.

1.2.4.3 Ostwald Ripening

As specified previously, Ostwald ripening follows continuously as soon as curved interfaces are present. The concentration gradient of the dispersed phase in the continuous phase causes larger particles to grow at the cost smaller particle. Ostwald ripening involves the movement of oil molecules from smaller droplets to larger droplets.

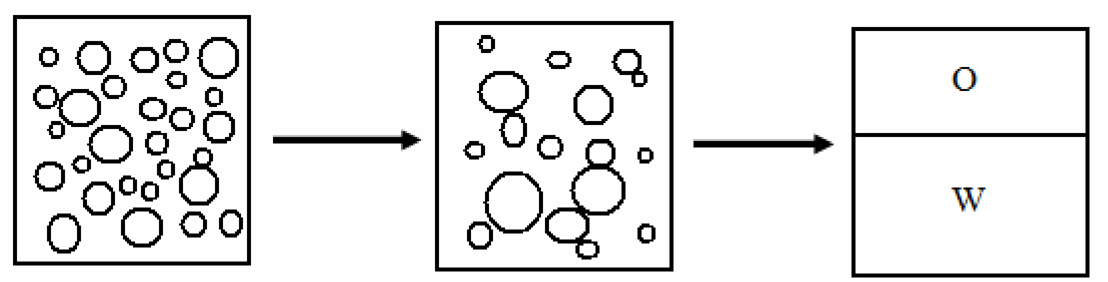


Figure 22. Seperation of the emulsion indicating the ostwald ripening process

The formation and stability of oil in water emulsion is directly connected with the transport of oil through the aqueous phase [21] and Liu et *al* [22] proposed that Ostwald ripening is always found to be one of the main mechanisms causing instability to emulsions especially nano emulsions.

Once steady state conditions have been attained, the change in mean droplet number diameter with time is given as:

$$d_i^3 - d_{t=0}^3 = {64\gamma DCC_{\infty} V_m^2 t}/{9RT}.$$

Equation *(9)*

The whole expression was given by Lifshitz–Slyozov–Wagner (LSW) model, where *t* is time, *d* is mean number droplet diameter, γ is the interfacial tension at the oil in water interface, *DC* is the diffusion coefficient of the oil in aqueous phase, $C_{\propto}$ the solubility of the oil in aqueous phase, V_m, the molar volume of the oil, *R* the gas constant, *T* the absolute temperature, and w the Ostwald ripening rate. Thus Ostwald ripening rate should increase as water solubility of the oil increases or the interfacial tension increases. The Ostwald ripening rate of an emulsion can be determined from the mean droplet size change (Figure 22) [21]. The main mechanism here is the mass transfer which is the diffusion of individual oil molecules between the droplets, the increase in droplet radius (r) with time where the equation above can be simplified as:

$$r_1^3 = r_1{}^3{}_{t=0} - \omega t \qquad \textbf{Equation (10)}$$

Here, ω is the molecular diffusion ripening rate. Considering the mass transfer mechanism, the increase in droplet radius with time is given by

$$r_1^2 = r^2{}_{t=0} - \omega_{mic}{}^t \qquad \textbf{Equation (11)}$$

Here ω_{mic} is the micelle-transport ripening rate. In large addition of surfactants after complete coverage of oil-water interface, surfactants form micelles in the continuous phase; these miscelles drastically increase oil solubility. Consequently effect of surfactant miscelles in the Ostwald ripening rate is always predicted. Nevertheless several studies report hardly any increase in Ostwald ripening rate by the effect of surfactant miscelles. Some researchers propose that mass transport is facilitated because of surfactant miscelles solublizing oil molecules in their hydrophobic interior and transport them across the aqueous phase separating the droplets [21]. Ostwald ripening occurs by the solubility of the oil phase in the aqueous phase and results in general coarsening of the emulsion. This process is mainly driven by difference in Laplace pressure between droplets having different radii. This solubility of the oil Phase in the continuous phase relates the radius of curvature of that droplet, the decrease in the radius with increase in solubility causing smaller drops dissolving in to bulk phase and then diffusing to, and redeposing upon layer ones leading to an overall increase in average size of the emulsion.

1.2.4.4 Effect of Viscosity

The stability of the emulsion depends on the viscosity of the continuous phase because of the influence of the viscosity on the diffusion of the droplets. A low diffusion constant reduces the number of collisions, so the rate of coalescence becomes smaller. For this reason, concentrated emulsions are often more stable than dilute ones, since the viscosity of the continuous phase rises with the number of droplets and therefore diffusion declines. The stability of the emulsion can be increased by stabilizing the forces between the droplets, by mainly the gelation of the continuous medium but this may change the character of the emulsion leading to the formation of more particle consistency.

If the inner phase of an emulsion occupies less than 30% of its volume, the individual droplets seldom interfere with each other. The viscosity of an emulsion change in accordance with the concentration of the dispersed phase, change in viscosity as a function of concentration can be described approximately with the Einstein formula

$$\eta = \eta_0 (1 + 2.5\ \phi) \qquad \textbf{Equation } \textit{(12)}$$

where, ϕ = ratio of volumes of the inner to the outer phase; η_0 = viscosity of the outer phase. In order to obtain a highly viscous emulsion in this condition, the outer or the continuous phase must have a high viscosity (the aqueous phase in the case of an o/w emulsion, a solvent immiscible with water in the case of a w/o emulsion). Polymer thickener also improves the viscosity. In water in oil emulsions, the water droplets are unstable and coalesce immediately, because the added polymer is restricted to the aqueous phase and does not form a protective barrier in the oil in water emulsions, the oil droplets dispersed in the aqueous phase are prevented from flocculation and subsequent coalescence. Thickeners soluble in the outer phase can be used to alter the viscosity as required. Common thickeners include cellulose derivatives, gelatin, casein, starch, dextrins, PVA (polyvinyl alcohol), PVP (polyvinylpyrrolidone), xanthan gum (Kelzan), carbopols (acrylic acid polymers), tragacanth, microcrystalline cellulose, and alginates [3]. When the dispersed phase occupies more than ϕ_1 >30% of the volume, the droplets begin to influence one another, and the viscosity increases up to $0.50 < \phi_1 < 0.52$. At higher values it escalates sharply, accompanied by non-Newtonian behavior. At $\phi_1 \simeq 68\%$, the emulsion is usually unstable unless a special emulsifier is added; this is the inversion point. At still higher inner- to outer-phase volume ratios, either the spheres adopt an even denser packing arrangement (honeycomb) or flatten out. The maximum possible occupation of volume is achieved at $\phi_1 = 74\%$. At $\phi_1 > 74\%$, the emulsion becomes polydisperse.

By increasing the viscosity of the dispersed phase, droplet disruption and droplet break up would be more difficult and so droplet diameter increases [18,23]. Increasing the continuous phase viscosity the recoalescence of the droplets is reduced because the drainage time between the droplets is extended by increasing the continuous phase viscosity while the collision time remains the same.

To systemically assess the stability and the properties of an emulsified preparation, the design requires the measurement of the droplet size, the determination of the zeta potential, microscopic analysis etc. If the emulsion contains an active substance, stability under storage at 4°C is relevant. Measurements carried out to determine viscosity and pH of the emulsion are necessary criteria for improving the quality control.

1.2.4.5 Effect of Ageing

On aging of emulsions, the viscosity decreases and shear-thinning effect also decreases. Up on aging substantial number of large droplets can be visualized. Further the process of ageing increases the mean droplet size and the width of the droplet size distribution. Coalescence would be the main reason for the coarsening of droplets, Ostwald ripening also do give rise to bigger size droplet formation by consuming smaller droplets and growing bigger. Thus ageing contributes to coalescence and Ostwald ripening and then ultimately phases separation. Hence the decrease in viscosity is likely due to increase in both the mean droplet size and the polydispersity. Figure 23 presents the stepwise change in droplet arrangement of a stable emulsion towards instability on ageing process.

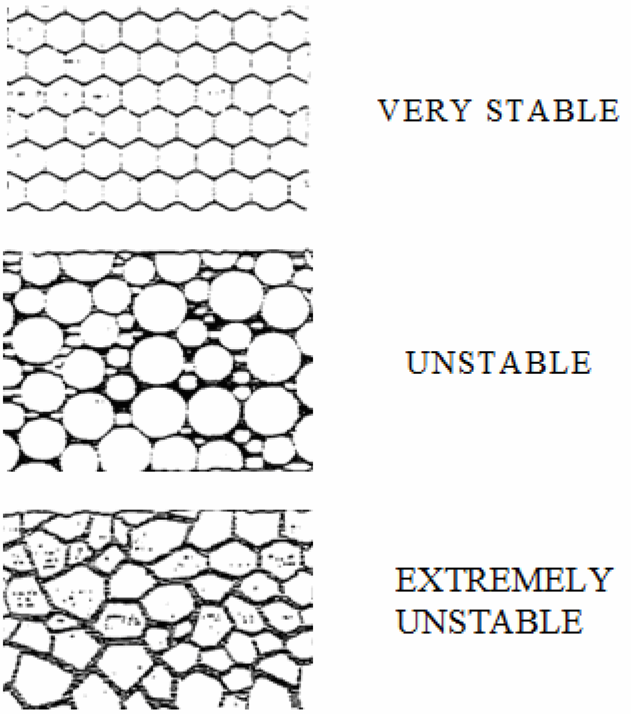


Figure 23. Ageing process

1.2.4.6 Particle size analysis

Particle size analysis is one of the important criteria to assess the stability of an emulsion. In this study particle size analysis was carried out using Laser Diffraction, Photon Correlation Spectroscopy (PCS), Phase Doppler Anemometry (PDA) and Microscopical analysis was also carried out. There are some Official Organizations that have published norms to deal with emulsions, namely AFNOR (Association Française de Normalisation) has published in 1976 a directive about Emulsion Analysis where the tests required for the stability studies are prescribed. [24].

1.2.4.7 Zeta potential

The emulsifying agent or the surfactant added to the emulsion to attain a stable emulsion will act as a barrier to alter the rate of coalescence of the droplets or creates an interfacial film which can produce repulsive electrical forces between the approaching droplets. A double layer is build up around the droplets after a monolayer of surfactant is absorbed, when an ionic surfactant is used; the water interface posses the charged segment of surfactant, the cations will be surrounding the anions. The counter ion concentration surrounding the anions is low in case, the thickness of the electrical double layer will be large and long- range repulsive forces will repel the droplets one another when they approach. The potential produced by the double layer creates a repulsive effect between the oil droplets and hinders coalescence [24].

Zeta potential is a quantity which can be determined to indirectly quantify the repulsive electrical potential at the emulsion interface.

1.3 Quality by Design

Quality by design relates directly with the Process Analytical Technology (PAT) Process Analytical Technology (PAT) is a system for designing, analyzing, and controlling manufacturing processes based on an understanding of the scientific and engineering principals involved, identification of the variables which affect product quality. The PAT initiative is consistent with the current FDA, that quality cannot be tested into products, but should be built-in by design. The primary goal of PAT is to provide processes which consistently generate products of predetermined quality. In so doing, improved quality and efficiency are expected from reduction of cycle times using on-, in-, or at-line measurements and controls prevention of reject product and waste, contributes to real time product release. It provides increased use of automation facilitation of continuous processing using small-scale equipment, resulting in improved energy and material use and increased capacity. Actually PAT is a system for designing, analyzing and controlling manufacturing [25], through timely measurements (i,e) during processing of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality. The quality can be improved by obtaining improved process and product understanding which is to identify and appropriately manage critical sources of variability and achieve "Right first time performance". The product quality and performance can be achieved by design of effective and efficient manufacturing processes. To design a process measurement system that allow on-line or at line monitoring of critical quality attributes and also to design a control system that allow adjustment of critical quality attributes and implementing a quality system that allows continuous improvement [26].

Adapting the improvement procedure for the measurement of particle size automatically concur implementation of Process Analytical Technology which promote the use of new analytical tools to facilitate a control-based approach over emulsification procedure. The implementation of Process Analytical Technology provides the adoption of real time analysis of the particle size which can be related with the outcome of the product. Unlike off-line measurements, online measurement offers a continuous stream of real-time data. The obtained data can be used to optimize the process operation [27].

In conventional practice, the final particle size is controlled as a mechanical operation which is not on-line thus the procedure is carried out after each operation prior to subsequent processing. This approach works; it is not, however particularly efficient. The manual input required for the analysis is very high. The product yield remains low and always not very well understood; there is frequently a high degree of product non-conformance.

On-line measurements:

PAT principally generates product quality information in real time. PAT centers on the use of in-line testing using near infrared, Raman and or other physico-chemical techniques as primary means of process monitoring. With the help of the probes associated in the process, uniformity and other targeted stages can be pinpointed to a high degree of certainty. Using PAT, processes would be under high control that results could be accurately predicted well before the product is analyzed. The manufacturing process could be continuously monitored and adjustments made to ensure that the finished product would meet the desired specification. This can be achieved by performing an online analysis of the process. In case of emulsification the online monitoring of the emulsification procedure paves way for the instant understanding of the procedure and how it is advancing, it gives a clear picture to derive at a decision that if the endpoint of the process would satisfy the requisites.

With on-line analysis many of the constraints associated with an off-line regime are removed, more data of higher quality is produced more rapidly. Concerns surrounding the representative nature of the samples, repeatability in terms of both sample preparation and analytical procedure, and frequency of analysis relative to process changes, are all eliminated.

On-line analysis allows the operator a real-time continuous view of the process that can be used to transform operational efficiency. This is more like an automated control. In an off-line analysis of emulsification, samples may be taken and measured every hour and the analysis may take 30-minutes. This indicates that the plant is controlled in an hourly basis as it gives hourly data that describes what was happening 30 minutes ago. Any deviation from the specification is rectified fairly slowly. The net result is poor plant control. In this study, oil in water emulsification was particle size analyzed on-line using Phase Doppler Anemometry. PDA can perform online particle size measurement providing ample feasibility to optimize the emulsion, now that it is easy to gain an understanding of how to control the process effectively. This gives a real confidence that the material produced will be in specification [27].

When we consider off-line measurements of particle sizing; over-processing is quite common. In order to produce an emulsion containing smaller droplets, the parameters like homogenization speed, surfactant volume, agitation time may be very easily altered or increased randomly without definite evaluation parameter. Phase Doppler Anemometry offers a better opportunity to alter or correct the required parameters by visualizing online the change during emulsification and from the signals which reflect the particle sizes, the formulator can act quickly and eliminate any drift away from his required specification. Since particle sizing is one of the parameter which governs the stability of an emulsion, the emulsion consistency, constancy can be derived out.

Online systems are becoming increasingly common in pharmaceutical industry like *Turbiscan online* which monitors and characterize emulsification process in real time, with a high acquisition frequency; *Malvern Insitec* which is laser diffraction based particle size analyzer. The presentation of Phase Doppler Anemometry for online analysis of particle sizes in emulsion is a better contribution which can be upgraded for a pharmaceutical purpose in spite of its complexity [27].

The evaluation kinetics of emulsification was studied using phase Doppler anemometry and also with Malvern zetasizer and Coulter LS 230. The PDA working on online evaluation of emulsion droplet size was focused importance. Prior investigations on phase Doppler Anemometry were done by various researches. Brenn et al. [28] employed PDA for the measurement of water and bubble velocity and diameter. The response of Phase Doppler system to spheroidal particles with different orientations was examined by Adrian Doicu et al. [29].

Phase Doppler Anemometry derives enormous pharmaceutical importance as this technique can be very well reached to the industrial scale for the online monitoring of the process of emulsification after major improvements acquired for the transition from the laboratory scale as this system is a laboratory instrument and it contains few major limitations.

1.4 Formulation Optimization

Optimization attributes to evaluation of the manufacturing process and to ensure that the process results in a consistent reproducible product is the primary concern. Optimization techniques tone down the helpless situation to predict the composition of appropriate emulsion. These techniques facilitate the product development. Any formulation requires an optimization process. The procedure involves defining of goals, evaluation procedures, compositions are defined, products should be prepared and evaluated appropriately and the formulation is modified until acceptable data are obtained. This in all probability involves a series of logical steps; the formulator should involve the rational procedures to control the variables until a satisfactory product results. But still the obtained product need not be an optimal formulation. In the main the optimization procedure consists of preparing a series of formulations, varying the concentrations of the formulation ingredients in some systematic manner. These formulations are then evaluated according to one or more attributes, such as particle size, particle size distribution, viscosity, volume ratio of the fractions in emulsions. Based on the results of these tests a particular formulation or a series of formulations may be predicted to be optimal formulation. Optimizing the formulation according to single attribute is relatively uncomplicated. To optimize on the basis of two or more attributes for example particle size distribution, viscosity may not be possible. The formulation that is optimal for one attribute very well may be different from the formulation needed to optimize other attributes. The final formulation is therefore is suitably modified to attain an acceptable performance of all relevant attributes [30].

Traditionally pharmaceutical formulations are developed by changing one variable at a time approach. The method is time consuming in nature and requires a lot of imaginative efforts. Moreover, it may be difficult to evolve an ideal formulation using this classical technique since the joint effects of independent variables are not considered. It is therefore very essential to understand the complexity of pharmaceutical formulations by using established statistical tools such as factorial designs [31].

Factorial designs are used in experiments where the effects of different factors or conditions on experimental results are to be elucidated. Factorial designs are the designs of choice for simultaneous determination of the effects of several factors and their interactions.

The physiochemical processes are multivariate with subtle interactions of variables. The techniques that can be used are Principal Component Analysis (PCA) and Partial Least Squares regression (PLS). They are recognized for their ability to eliminate noise and identify latent variables [30].

Principal Component Analysis, a multivariate statistical procedure, was used by Bohidar and group [32]. This method determines the interrelationships and covariance among the measurements employed to evaluate the adequacy of a formulation. That is it identifies the variables that best distinguish one formulation from another and which should be the criteria used in product development [32]. Using a selection method one can simplify the optimization procedure by discarding those responses which are highly correlated with other responses. Principal Component Analysis (PCA) can be used to select these key responses [33]. Solè et al. [34], used Central composite design to optimize preparation variables such as mixing rate and stirring rate of the oil in water nano emulsions formed in the system W/ Potassium oleate - oleic acid- $C_{12}E_{10}$/ hexadecane at constant composition, the author explains that experimental design permits one to obtain a quadratic equation for the droplet diameter, (i,e):

$$\text{Droplet diameter (nm)} = a + b\,(\text{addition rate}) + c\,(\text{mixing rate}) + d\,(\text{addition rate})^2 + e\,(\text{addition rate})(\text{stirring rate}) + f\,(\text{mixing rate})^2 \quad \textbf{Equation } (13)$$

However, taking in to account only the significant parameters at 95% confidence levels, the equation can be reduced as:

$$\text{Droplet diameter (nm)} = a + b\,(\text{addition rate}) + c\,(\text{mixing rate}). \quad \textbf{Equation } (14)$$

Here occurs no quadratic term in the equation, so the droplet size has a linear dependence with variables, stirring rate and mixing rate which are independent. The smallest droplets can be obtained at high mixing rates and low addition rates as proposed by the author. Further mentioned that mixing rate controls the emulsification path and increased agitation rates with slowest addition rates are important for high viscous dispersed phases.

Phase diagrams outlining the behavior of multiple components have been extensively utilized in formulation optimization in spite of the large amount of data that must be obtained if adequate diagrams are to be prepared.

Variants of these geometric representations include Simplex lattice procedure that has been employed to determine the optimal composition of the formulation with respect to certain variables or to a specific outcome.

In the absence of rigorous approach to optimization, the product may turn out to be a formulation with a sub-optimal character due to instability. The formulation optimization can be achieved by the application of Process Analytical Technology. In this study, the oil in water emulsification is optimized by the application of PAT.

In order to optimize the oil in water emulsion, a greater understanding is required and improved process control over emulsification is necessary. Thus the formulation can be optimized and each time it is manufactured by on-line analysis of the emulsification process; this provides a very detailed view of a process that translates directly in to improvement in the highest of product quality. Under the section 1.5 various online measuring techniques for the droplet size determination have been discussed.

1.5 Measurement Techniques

1.5.1 Particle measuring techniques

Under this section, the Particle measuring techniques are explained, although many of these techniques were not experimented, they are included as they seemed promising. The on-line techniques can provide real time analysis of particle size and concentration, enhancing process control and quality assurance of the product stream. The large number of laser-based systems, suitable for laboratory use, both *in-situ* and off-line, as well as industrial, on-line instrumentation provide many options for particle-size analysis. Figure 24 represents various techniques for the particle measurement, from which some of the interesting techniques were explained.

Ensemble methods of particle sizing measure the distribution of a group, or ensemble of particles. Most methods falling in this category use Fraunhofer diffraction to describe the underlying physics on which measurements are made. The instruments such as the Malvern particle analyzers, the Coulter LS series fall under the above mentioned category and the majority of these instruments are designed for off-line instruments suitable for work in the laboratory as bench-top units. Single particle counters measure individual particles where they are of two types one is dependent on the amplitude of the scattering light and the other is independent on the amplitude of the scattered light. The discussed Phase Doppler anemometry falls under the amplitude independent method, other examples are sedimentation, impaction based studies and photographic methods of image analysis and methods using microscope.

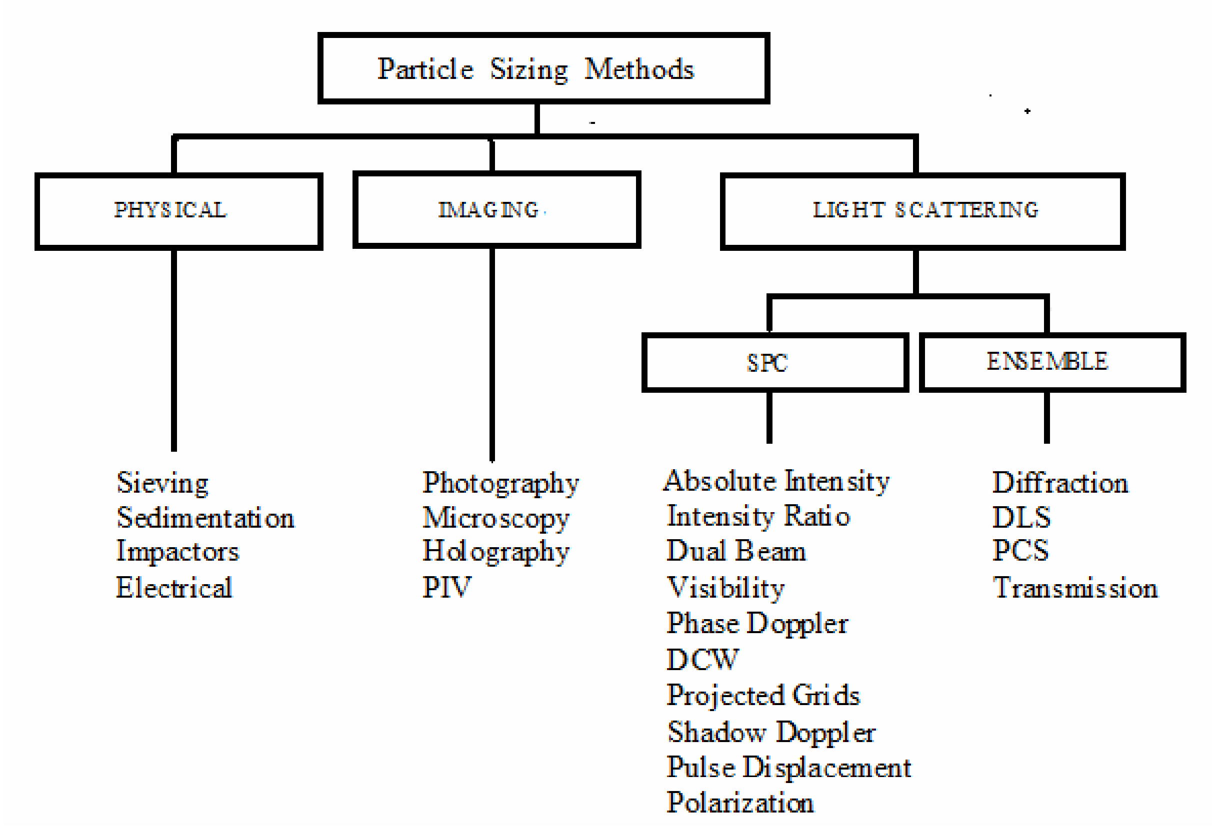


Figure 24. Representation of different techniques of droplet size measurement [35]

1.5.1.1 Video enhanced Microscopy

Video enhanced microscopy is a technique that combines the magnification power of a microscope with the image acquisition capability of a video camera [36].The resulting data matrix, from which information about the sample can be extracted, is an image or a series of images. The distinctive information that could be obtained in images in sample state, geometry, dispersity etc. when we consider emulsions on this regards this generally implies droplet size and concentration which are important properties of any emulsion. Here series of images or continuous video provide information about droplet interactions and kinetics of important processes within the emulsion, like flocculation and coalescence. These parameters are central to the understanding of emulsion behavior and emulsion stability. Usually it is facilitated with a computer. The system as it is computer aided video enhanced light microscopy. The Figure 25 shows the schematic representation of the video enhanced microscopy system.

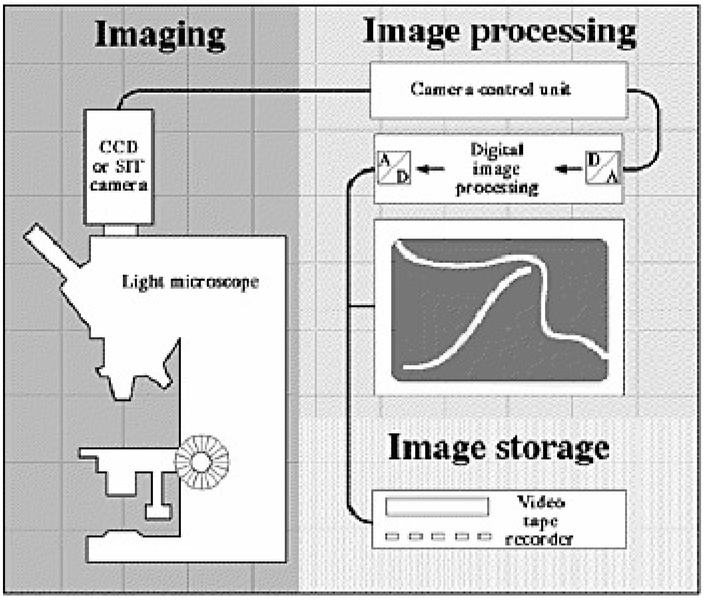


Figure 25. Representation of video enhanced microscopy [38]

Here the microslide is used which is a thin flat rectangular micro capillary of glass mainly useful for preparation of liquid samples vulnerable to evaporation of shear. The computer aided video microscopy performs series of three different functions such as Imaging, Image processing [37] and Image storage. The images are recorded by a video camera system which is a low light level charge coupled device [CCD] or silicone intensified target [SIT] camera for fluroscence light microscopy and generate signals that can be enhanced in various ways by analog and digital image processing. The camera control unit is an anolog enhancer that allows grey level selection and gain manipulation to optimize the image [38]. Digital image processors convert the anolog to a digit signal and can store images for further process like background subtraction in order to compensate for uneven illumination and to compute moving averages to optimize image contrast. The processed digital images are re-converted in to an analog signal that can be displayed on a video tape. Holt et al. [39], confirmed the applicability of video enhanced microscopy (VEM) to the investigation of reversible coagulation and evaluation of doublet lifetimes in emulsions. This method scores over the turbidity measurements. The accuracy of the measurement can be improved by the preparation of emulsions with low concentrations of 1µm and submicron droplets. The discrimination between 1 and 2 µm droplets is difficult which decreases the reproducibility of the experimental data.

1.5.1.2 Photon correlation spectroscopy

Dynamic Light scattering is also termed as Photon correlation spectroscopy, quasi elastic light scattering etc. Light scattering methods measures the time-dependent fluctuations of scattered light are termed as dynamic light scattering, quasi elastic light scattering (QELS) or photon correlation spectroscopy (PCS). The term dynamic light scattering (DLS) indicates that the method gives information on the movement of the scattering particle. The scattering of photons by the moving particles is quasielastic; the term quasi elastic light scattering is used as well. Most dynamic techniques employ autocorrelation, hence the term photon correlation spectroscopy (PCS).

Dynamic light scattering is a technique which considers Brownian motion to measure the size of the particles. Brownian motion is the random movement of particles due to the bombardment by the solvent molecules that surround them. This Brownian motion of the dispersed particles causes fluctuations in the total intensity of scattered light which are related to the velocity of the particles. Since the larger particles move less rapidly than the smaller ones, the intensity fluctuations yield information on the particle size. The larger the particle, the slower the Brownian motion will be. The velocity is given by the diffusion coefficient (D). The velocity of the scatterer is measured in order to calculate the diffusion coefficient. The particle size can be obtained from diffusion coefficient [40].

The size of a particle is calculated from the diffusion coefficient by using the Stokes-Einstein equation;

$$d(H) = kT/3\pi\eta D \qquad \textbf{\textit{Equation}}\textit{(15)}$$

Where d(H) is the hydrodynamic diameter, D is the diffusion coefficient, k is the Boltzmann's constant, T is the absolute temperature and η is the viscosity. The diameter of the particle measured in PCS is a value that refers to how a particle diffuses within a fluid so it is referred as a hydrodynamic diameter. The diameter obtained is the diameter of a sphere that has the same translational diffusion coefficient as the particle. The upper size limit is set by the onset of sedimentation in the sample, this usually dependent on the density of the sample. The lower size limit depends on sample concentration, refractive index, laser power and sensitivity of the detector.

An accurately known temperature is required for PCS as it is necessary to know the viscosity accurately (viscosity of the liquid is related to the temperature). The temperature has to be stable as well if not the convection currents in the sample will cause non-random movements which will ruin the interpretation of size [41].

In photon correlation spectroscopy, the intensity fluctuations of scattered light arising from the particles are measured. From a system of particles undergoing Brownian motion, a speckle is seen to be in constant motion. This occurs because the phase addition from moving particles is constantly evolving and forming new patterns. The rate at which these intensity fluctuations occur will depend on the size of the particles (Figure 26).

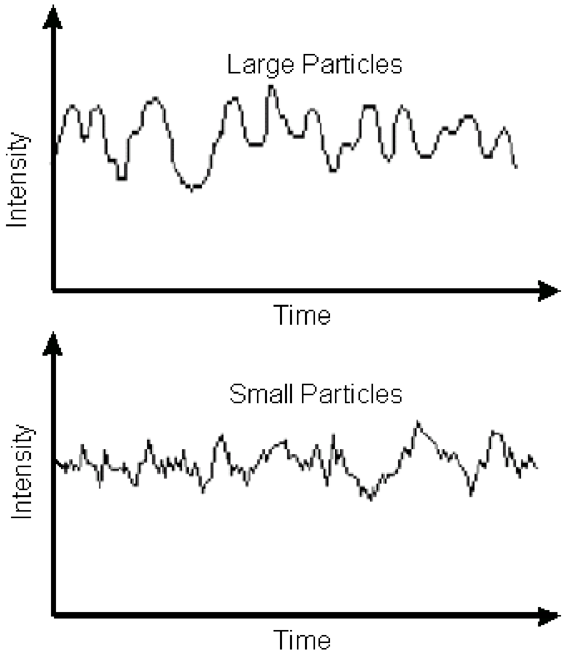


Figure 26. Intensity fluctuations from the dispersion

The Brownian movement can be interrupted by the sedimentation or particle-particle interactions, in a system where there occurs no sedimentation or particle-particle interactions the movement of the particle is random. The fluctuation intensities differ very much in time, thus fluctuations observed after a large time interval do not resemble the initial fluctuation pattern. When there exists a time differential between the observations is very small such as nanosecond or a microsecond, both the position of the particles is similar and also the scattered intensities are correlated. Increase in time interval causes decrease in correlation. The decay of correlation is size-dependent. The smaller the particles are, the faster is the decay. The smaller particles cause the intensity to fluctuate more rapidly than the large ones. The fluctuations in scattered light intensities are detected by a photomultiplier and are recorded. The data containing information on the particle motion are processed by a digital correlator. The correlator compares the intensity of the scattered light at time t to the intensity observed at a very small time interval (τ).

1.5.1.3 Laser diffraction

Laser diffraction can be more appropriately called Low Angle Laser Light Scattering (LALLS). This technique has become the preferred standard in many industries for characterization and quality control. The applicable range is 0.1 – 3000μm. Instrumentation has been developed in this field over the last twenty years or so. The foremost reliability of this method is on the detail that diffraction angle is inversely proportional to particle size. Diffraction-based particle analyzers pass a laser beam through a sample (dispersion). The light scattered from all particles in the sample volume is collected with a Fourier transform lens and focused on to a detector, or series of detectors, of suitable geometry to characterize the angular separation of the incoming scattered light. A Fourier transform lens describes a standard lens used in such a manner that the angle of the incident scattered light corresponds to a particular annular position in the focal plane of the lens, regardless of the position of the scattering particle. The Fourier lens forms the moving diffraction patterns of the particles traversing the sample cell to change in to scattering composite diffraction pattern that can be measured by a stationary set of detector arrays. The composite time-averaged diffraction pattern is used measure the droplet size distribution. Instruments consist of laser as a source of coherent intense light of fixed wavelength. He-Ne gas lasers (λ=0.63μm) are the most common as they offer the best stability (especially with respect to temperature) and better signal to noise than the higher wavelength laser diodes. The detector used is a slice of photosensitive silicon with a number of discrete detectors. It is said that better with best possible number of detectors say 16 to 32 but increased numbers do not mean increased resolution. When we consider photon correlation spectroscopy technique (PCS) used in the range 1nm – 1μm approximately, the intensity of light scattered is so low that a photomultiplier tube, together with a signal correlator is needed to make sense of the information. Particles in suspension can be measured by recirculating the sample in front of the laser beam [42]. Older instruments and some existing instruments rely only on the Fraunhofer approximation which assumes:

-particle is much larger than the wavelength of light employed.

-all sizes of particle scatter with equal efficiencies;

-particle is opaque and transmits no light.

When the particle size approaches the wavelength of light the scattering becomes a complex function with maxima and minima present. The latest instruments (e.g. Mastersizer 2000, Malvern Instruments, and Coulter Counter) use the full Mie theory which completely solves the equations for interaction of light with matter. This allows completely accurate results over a large size range (0.02 -2000μm typically). The Mie theory assumes the volume of the particle as opposed to Fraunhofer which is a projected area prediction. The "penalty" for this complete accuracy is that the refractive indices for the material and medium need to be known and the absorption part of the refractive index known or guessed. However, for the majority of users this will present no problems as these values are either generally known or can be measured.

1.5.1.4 Phase Doppler anemometry

Laser Doppler spectroscopy and phase Doppler anemometry are relatively new methods for particle sizing. The Laser Doppler anemometry was used for determining the translational diffusion coefficients of macromolecules. They measure the flow of the particles.

The interest in particle size measurement became a major aspect after the onset of the development of the phase Doppler anemometry. Laser Doppler anemometry was modified and development let to the possibility of obtaining the size information. The phase Doppler anemometry with its variations becomes a major sizing tool. Laser Doppler anemometry, phase Doppler anemometry, particle image velocimetry, particle tracking velocimetry, multipoint number fluctuation laser velocimetry all these techniques characterize particles by their light scattering during motion [42].

Doppler effect

As indicated by the name Laser Doppler anemometry, the Doppler effect plays an important role in LDA. This technique is mainly the Doppler shift of the light reflected from a moving seeding particle.

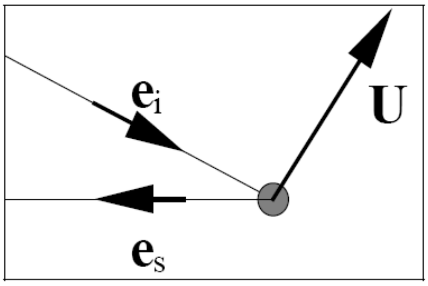


Figure 27. Scattering of Light from a moving seeding particle

The principle of the Doppler effect is illustrated in the Figure 27, here the vector **U** represent the particle velocity, the unit vectors e_i and e_s explain the direction of incoming and scattered light respectively. The incoming light has the velocity c and the frequency f_i, but due to the particle movement, the seeding particle has a different frequency f_p, which is scattered towards the receiver. Thus from the receivers point of view, the seeding particle act as a moving transmitter, and the movement introduce additional Doppler shift in the frequency of the light reaching the receiver [43]. Lorenz-Mie light scattering theory is applied when the particle sizes are comparable to the wavelength of the light. This theory considers the spherical particles, and thus describes only the dependency on particle size. Larger particles scatter more light than smaller ones.

The phase Doppler anemometer is nothing but a LDA with two optical receivers for particle sizing that measures simultaneously the size and the velocity of the particle. The phase Doppler anemometry is a single scattering technique and is a single counting method; only one particle is assumed to be present in the measurement probe volume at a time. Studies

[44], have shown that discrete Fourier transformation processor can be used to simultaneously measure the size and velocity of the two particles. Phase Doppler anemometry is based on the phase difference of the light scattered from individual particles. Two laser beams intersect at an angle forming the measuring volume. A spherical particle passing through the measuring volume scatters the light. Two burst signals detected under different elevation angles have a phase shift which depends on particle size, composition and the geometry of detection. Using this approach, the response function of a PDA instrument can be given by the following linear relationship:

$$d = \frac{\lambda}{2\pi F}\phi$$ ***Equation*** [16]

In the equation, λ is the laser wavelength, Φ is the phase shift between any two detectors and F is a function of the optical configuration of the system and the relative refractive index (N) between the particle and the medium.

The phase shift caused by particles, much larger that the wavelength of the incident light can be described by geometrical optics. For particles smaller than 10 μm geometrical optics is not applicable and the Lorenz-Mie theory is employed for signal processing.

In PDA, the difference in optical path length for the reflections from the two incident beams change with the position of the photo-detector, that is when the particle passes through the measuring volume, both the photo-detectors require a Doppler burst of the same frequency, but the phases of the two bursts vary with the angular position of the detectors (Figure 28).

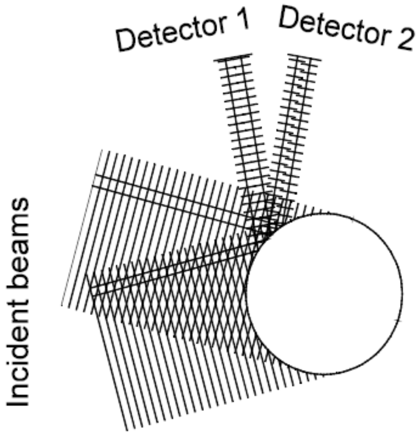


Figure 28. Interference patterns differ at the two photo-detector surfaces

The Instruments include Ferometrics, Dantec, Invent and TSI. The simultaneous measurement of velocity (up to three components) and size of spherical particles as well mass flux, concentration etc. Phase Doppler anemometry is the first commercial instrument launched in 1984 which provides highly non-intrusive measurement. The main advantage is PDA technique is applicable for online measurement and in site measurements. No calibration is required which is an absolute measurement technique. PDA proves a very high accuracy and very high spatial resolution, with a small measurement volume. The measurement of sub micrometer particles by Doppler principle is restricted by fundamental considerations. The phase difference between signals for small particles is very small and different to measure even with the most sensitive optical systems.

1.5.1.5 Modified spatial filtering technique:

This technique is the combination of Spatial filtering velocimetry (SFV) and Fibre-optical spot scanning (FSS). This method measures the velocity and size of the droplets simultaneously [45]. This inline measurement technique provides better understanding of the particle systems and improves the control of processes. The particle size measurements can be divided in to two categories, stream scanning and field scanning. Stream scanning contributes to the examination of one particle at a time and field scanning leads to simultaneous examination of an assembly of particles. Both the principle deserves a prime application on particle sizing of industrial processes, leading to on-line size analysis. The velocity and size of the droplets can be obtained as the droplets pass through a Laser beam and cast shadows on to the optical fibers. This technique actually does the measurement of the time length that a particle interrupts a light beam and how rapidly it consecutively blocks a series of detectors is recorded. The particle chord length is measured from which the particle diameter can be obtained. Spatial filtering velocimetry is mainly the method of determining the velocity of an object by observing it through a spatial filter in front of a receiver. The fiber-optical spot scanning (FSS) is an addition to the spatial filter to obtain a modified spatial filtering technique, the basic operation of the FSS is to observe the shadow image of a moving particle through a single optical fiber with a small diameter.

When the shadow image passes through the single optical fiber, an impulse is generated, the width of which depends on the particle size, particle velocity and random location of particle and fiber [46]. The probe with the modified spatial filtering technique uses a fiber-optical configuration which is shown in the figure. The single fiber for spot scanning and a fiber-optical spatial filter are arrayed together. The shadow of the moving particle is formed at the entrance faces of a differential-type optical fiber spatial filter with intervals.

Fiber-optical spatial filtering velocimetry was modified by fiber-optical spot scanning in order to determine the particle size. The in-line measuring device require the use of the probes IPP30 and IPP50, based on a modified spatial filtering technique the probes are available with measuring ranges of 10 ± 2500 μm and 0.01 ± 20 m/s with a data rate of 500 s^{-1} [47].The advantages of this in-line measuring device are the low hardware requirements, long-term stability and the robust design.

1.5.1.6 Back scattering of light

Light back scattering method contributes to the factor allowing the measurement of particle size distribution in dense dispersions. This technique finds its application mainly in process streams, monitoring suspension polymerization, and for the determination of aggregate size during flocculation. Back scattering differs from the forward scattering as it is mainly determined from the single particle by reflection at the particle medium interface and multiple inter particle reflections and not by the diffraction [42].

Back scattering technique applies to the optical analyzer, Turbi Scan MA 2000, which is the equipment used for the measurement of the particle sizes. The heart of Turbiscan MA

2000 is a detection head which moves up and down along a flat-bottomed glass cylindrical cell.

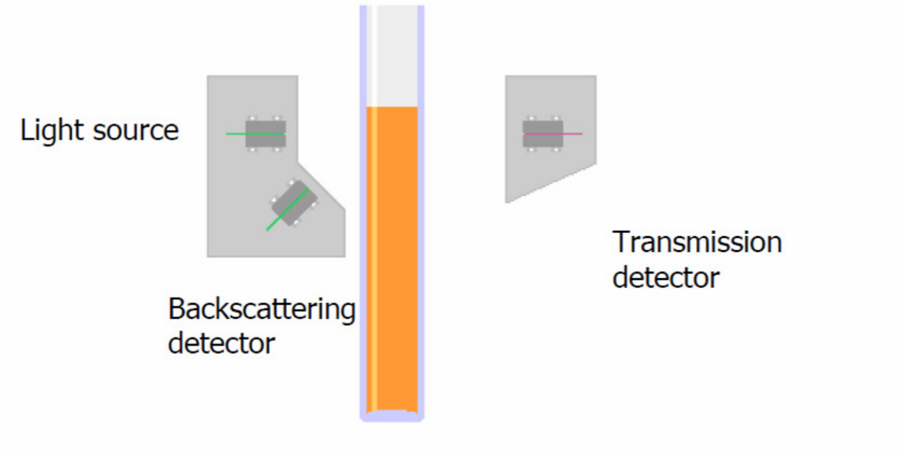


Figure 29. Principle of multiscattering technique (Turbiscan®) measurement

The detection head composed of pulsed near infrared light source (λ = 850 nm) and two synchronous detectors. The transmission detector receives the light which goes through the sample which would be at about 0° from the incident beam. The optical sensor consists of a pulsed near- infra red light transmitted through the emulsion or suspension and back scattering detector receives the light scattered by the sample 135° from the incident beam(Figure 29). The detection head scans the entire length of the sample (about 65 cms) acquiring transmission and backscattering data each 40 µm producing 1625 transmission and backscattering acquisitions per scan. The optical sensors acquire transmission and back scattering signals from 0.1 to 10 seconds every 40 µm along the sample tube for a maximum of 80 mm, and these signals are digitized and displayed by the software indicating real-time changes in transmission and back scattering intensities. These parameters are directly related to particle size and volume concentration. Mengual et al. [48], suggests that multiple light scattering method (Turbiscan MA 2000) allows to detect two kinds of destabilization phenomena like particle migration (creaming, sedimentation) and particle size variations (coalescence, flocculation). The monitoring of the sedimentation of the sedimentation front of the concentrated suspensions can be obtained and there by the particle and aggregate size can be estimated. This study also suggests that compared with other optical analytical methods such as microscopy, particle size and zeta potential analysis, the optical method presents the advantage of being a non-destructive tool as there is no sample dilution. Further studies [49] on stability of the emulsions also suggest that, optical scanning analyzer (Turbiscan) facilitates flexibility and being an absolute tool, which enables to study in depth the stability of many different pharmaceutical products in order to improve the formulations regarding stability.

Focused Beam Reflectance:

Another in-situ, laser based measurement technique which uses light back scattering effects is the Focused Beam Reflectance Method (FBRM). The principle of the cylindrical FBRM probe, which can be easily installed in stirred tanks or pipes, is shown in Figure 30. This can be easily located within the emulsions to be characterized [45]. The optic, the infrared laser beam rotates at a high velocity and focuses the laser beam near to the sapphire window. Passing particles backscatter the laser beam so that the chord length of the particle is computed by multiplying the scanning time (t) with the beam speed (v_b). As the optical signal is processed by the device electronics and the corresponding chord length is calculated as the product of the measured crossing time and beam velocity. A chord length is a straight line between any two points on the edge of the particle (or particle agglomerate). Typically, up to 100 000 chords are measured per second, which results in a chord length number distribution. Chord length counts are summed up in a finite number of chord length intervals and thus yielding Chord length distribution. Usually, thousands of chord lengths are measured each second. This allows determining a robust chord length distribution, which can be used to illustrate changes in particle dimension, particle population and particle shape in time.

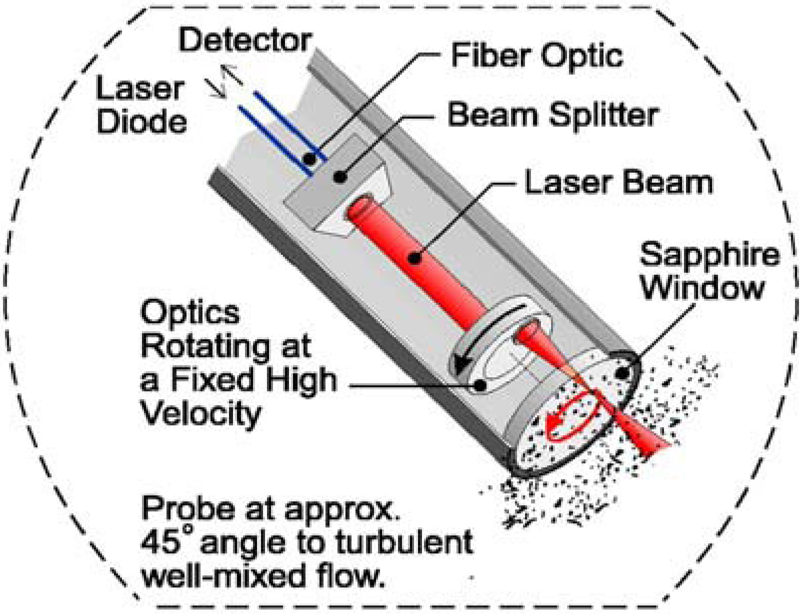


Figure 30. Schematic representation of FBRM probe (Lansantec®) for in-situ analysis

Ruf et al. [50], explained the position of the probe window with respect to the flow of the dispersion is of key importance and that an increase in flexibility of the signal processing of FBRM device will probably allow more accurate measurements. The author substantiate that this technique can be used online and in situ without sampling and dilution of the sample where he compared the measurement of ceramic and glass spheres by using HELOS (Partice size analyzer with dry Laser diffraction measurement; size range 0.1 µm to 8750 µm) with FBRM analysis. This comparison demands more explanation as the direct comparison between HELOS, a dry laser diffraction method and FBRM measurement in suspensions is questionable as no quantitative value can be attributed to it.

Dowding et al. [51], showed the possibility of making in-situ, real time droplet size measurements over a wide range of droplet volume fractions using FBRM analysis unlike in many other techniques which demands dilution which may result in changes in droplet size distribution associated with break-up/ coagulation or coalescence. In his study parameters like stabilizer effect, stirrer speeds were considered. PVA stabilized oil in water emulsion droplets comprising styrene, DVB, methyl pentanol and benzoyl peroxide with an increase in stirrer speed resulted in the reduction of chord length of droplets obtained, without any significant change in the width of size distribution. Changes in stirrer design were found to only have a small effect on the size/width of size distribution of droplets formed. An increase in polymeric stabilizer concentration results in a slight reduction in the average size of larger droplets. The droplets so formed are, however, more stable. If small amounts of polystyrene are dissolved in the oil phase, larger droplets are significantly more stable to break-up.

1.5.1.7 Endoscope technique

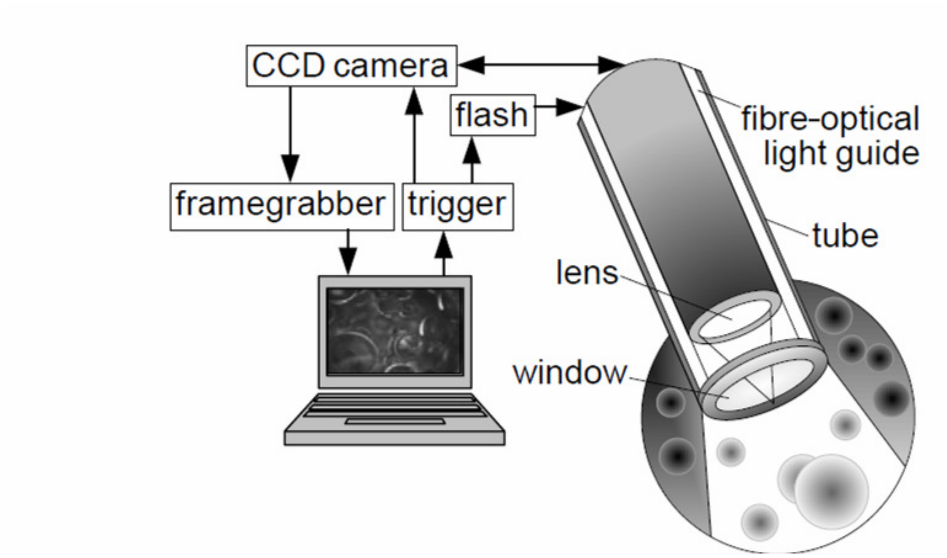


Figure 31. Schematic representation of the endoscope technique

In the endoscope technique images are taken from inside the vessel, this technique is intrusive; the endoscope which is 7mm in thickness is placed in front of the CCD camera as a microscope lens. To avoid disturbances by drops in front of the focal plane a covering tube with a window is placed at the tip of the endoscope lens. However minimal influence occurs on the flow pattern. A strobe flash is guided by a fibre optic cable surrounding the endoscope to ensure sharp pictures even in vicinity of the stirrer where speeds up to 1m/s are reached [45].

The drops are semi-automatically measured and counted as the counting has to be done manually at a pace around 400 drops an hour. The technique is capable of measuring drops of 25μm to 1000μm at any dispersed phase fraction. This technique offers reliable in-situ measurement of drop sizes from any part of the mixer vessel. The endoscope technique facilitates the ability to analyze the high dispersed liquid-liquid systems quantitatively.

1.5.1.8 Sedimentation Techniques

Sedimentation method of determining the particle size distribution is done by measuring the sedimentation velocity of particles settling under the influence of gravitational or centrifugal forces. Measurement of the sedimentation velocity in an initially homogeneous dispersion is quite easy. A special method can be introduced called the Line-start method where a thin layer of the dispersion is placed on the sedimentation fluid and the settling of the particles is measured. Larger particles settle faster than the smaller ones [42].

Main principle under the sedimentation methods is that the settling of the particles obeys the Stokes' law according to which the particle size to the sedimentation velocity is proportional to the square of the particle diameter and the difference in the specific density of the particle and the liquid which can be given as:

$$\frac{dh}{dt} = \frac{2(\rho_1 - \rho_2)\, r^2 g}{9\eta}$$ which is the ***equation*** 2

where *dh/dt* is the change in height with time, ρ_1 and ρ_2 are the densities of the phases 1 and 2.Multi sample analytical centrifugation is a useful tool in contributing high versatility in characterization of emulsion stability; this centrifugal sedimentation method is the unique method where the measuring scheme is pictured in the Figure 32 [52].

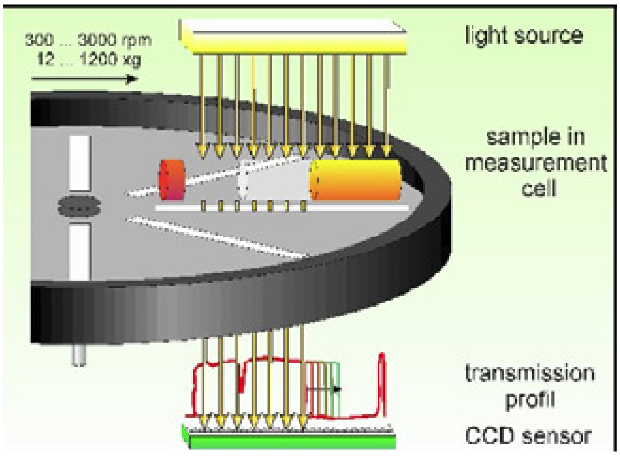


Figure 32. Representation of Analytical Centrifugation

Near infra red light is used and passed parallel through the sample cell and the distribution of the local transmission is recorded over the entire sample length at preset time intervals. At this time intervals the transmission distribution is determined over the whole sample length, ample number of samples can be analyzed simultaneously. Droplet migration due to centrifugal force or change in size distribution results in a variation of the local droplet concentration and corresponds in an alteration of the transmission. The progression of transmission profiles contains the information on stability and kinetics of separation processes.

Sedimentation by gravity is limited to particles larger than 5µm in diameter, smaller particles take sufficiently long time to settle and here Brownian movement may interfere with the settling of smaller particles. The stokes law can be applied only under the condition which supports unhindered sedimentation of spherical particles, moreover the flow should be a laminar flow and also the particles move without interference and particle should be spherical and rigid. The sample need to be diluted to a greater extend. The accuracy of particle sizing depends on validity of stokes law for a given system.

1.5.1.9 Discussion and comparison of methods

It is always critical to be cognizant of the advantages and disadvantages of the methods mainly suitability of each technique for the system to be measured. The main parameters to be considered are as follows:

-range and distribution of the particle size;

-shape of the particles;

-time required for the measurement;

-cost of the equipment and the analysis.

Particle size measurement values obtained by different methods are likely to differ. The main reason behind this is because the assumptions made for calculating the particle size may not be valid for the sample measured. After comparing the techniques a common fact can be concluded that the particle sizing techniques assume particles as a spherical one but most dispersion are irregularly shaped [42].

Hunt and Wolf measured the particle size distribution of glass beads using Coulter Counter, electroformed sieves, a microscope, sedimentation technique [53]. According to this author particle size measurement values of Coulter counter and Microscope goes hand in hand, sieve method and microscopy differs a lot. Sedimentation showed a broader size distribution than microscope results. Dynamic light scattering methods showed better outcomes except for the wide size distributions. Light diffraction method found to be less suitable for sub micrometer range. Studies about the influence of particle shape on determination of particle size using Laser diffraction, Sedimentometry reveals that the methods showed good agreement for spherical particles but considerable discrepancies for the irregular particles. Sedimentation results were in close agreement even though the method is size dependent [42]. While there are so many different possibilities for sizing drops in stirred liquid/liquid dispersions an evaluation for specific applications is necessary. The Focussed Beam Reflectance method (FBRM) and the endoscope technique seem to be the most promising techniques. Maaß et al. [45], have compared the FBRM and endoscope technique by experimenting the system toluene/water at pH = 7 with varying stirrer speed and dispersed phase fraction. The respective probe is introduced into the tank close to the stirrer at the same position to eliminate influence of the local position. Transient drop size distributions were measured for each parameter combination for about one hour. The author proposed that with the endoscope it was possible to analyze single drop sizes and data obtained were reliable and concluded that for detailed information about the accurate drop sizes of the analyzed system only the endoscope technique was useable.

Main problem that exist with the particle sizing methods is fundamental dependence of accuracy on Particle shape, particle size distribution and particle-particle interactions.

1.6 Objective and scope of the study

The main approach is the dynamic evaluation of droplet size distribution of emulsions. Various methods employed in the measurement process were discussed. The ultimate goal is to study the emulsification kinetics. Here Phase Doppler Anemometry is applied for the evaluation kinetics of the oil in water emulsion. The PDA contribution for the online measurement of emulsion droplets can be consociated with the application of PAT in the process of emulsification.

The process of emulsification is based on a complex mechanism. In order to provide information instantly during the process of emulsification, observation and quantification of emulsion kinetics demands vital importance. Process Analytical technologies should help on assessing the quality of an emulsion instantly. PAT facilitates on optimizing the emulsion formulation and processing. To acquire an overall understanding of the different particle size measurements, a detailed study is carried out. In this case, the on-line particle size analysis is a major tool to facilitate the optimization of the formulation [54], the kinetics of o/w emulsification is studied and the online monitoring of emulsification is carried out. Chemometric procedures required for optimization of the formulation does assessing the formulation and its kinetics online helps in huge degree to optimize the product to a very high quality. For the study of the kinetics of an oil in water emulsification process inclusion of the active drug ingredient is not essential as the main focus of the study is established to the online monitoring of the emulsion formation, how to improve the scope of the emulsion during the process of emulsification. This approach is followed in all industries with the help of PAT techniques so on this basis, Phase Doppler anemometry is chosen and aimed to extend its contribution to online monitoring of Particle sizing. In spite of few limitations, PDA techniques also reach to the requirements for the optimization; it is studied to measure the particle size distribution online.

Chapter 2 MATERIALS AND METHODS

2.1 2.1Materials

2.1.1 Liquid Paraffin

2.1.1.1 Description

Liquid paraffin is a viscous liquid with a kinematic viscosity of 212.5 m Pas. The Paraffin can be a liquid with increasing viscosity (C_5 to C_{16}) [55]. The density ranges from 0.827 to 0.890 g/ml at a temperature of 20ºC. The refractive index is 1.4754. The molecular weight range is about 200.15 g/mol. (information provided by the supplier)

Liquid Paraffin, batch no: 07211231, was supplied by José M. Vaz Pereira, S.A, Portugal.

2.1.2 Polysorbate 80

2.1.2.1 Description

Polysorbate 80 (Tween 80™), is an nonionic surfactant derived from polyethoxylated sorbitan and oleic acid. Polysorbate 80 is a viscous, water-soluble yellow liquid. The hydrophilic groups in this compound are polyethers also known as polyoxyethylene groups which are polymers of ethylene oxide. The hydrophilic lipophilic Balance value is 15. The density ranges from 1.06 – 1.09 g/ml at a temperature of 20ºC. The refractive index is 1.4728. The molecular weight is 1310 g/mol. (information provided by the supplier)

Polysorbate 80 (Tween 80™), batch no: A0249698 was supplied by Acros Organics, New Jersey, USA

2.1.2.2 Structural formula

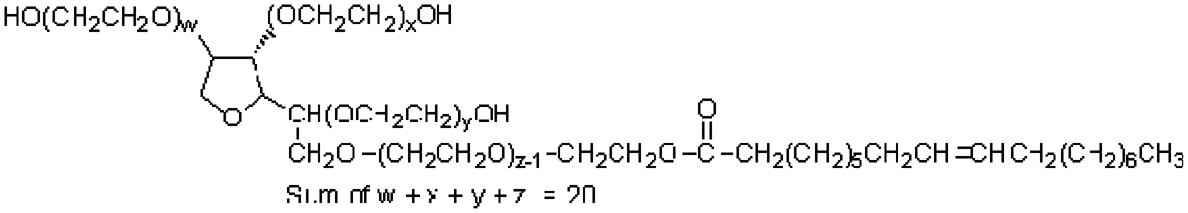


Figure 33. Polysorbate 80

2.1.3 Sorbitan Monolaurate.

2.1.3.1 Description

Sorbitan monolaurate (Span 20™) is slightly yellow to deep brownish yellow in color. It appears as clear viscous liquid, also behaves like a paste. The Hydrophilic Lipophilic balance value of 7.6 to 9.6. The molecular mass is 346.46, the molecular formula is $C_{18}H_{34}O_6$. The density of Sorbitan monolaurate is 1.032 g/ml at a temperature of 25°C. (information provided by the supplier)

Sorbitan Monolaurate (Span 20™), batch no: 0000051747 supplied by Panreac Quimica, Spain

2.1.3.2 Structural formula

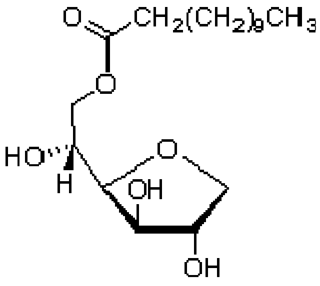


Figure 34. Sorbitan Monolaurate

2.1.4 Water

The dimineralized water was supplied from Milli-Q apparatus (Millipore Corporation, USA)

2.1.5 Equipments used

2.1.5.1 Ultra-Turrax (UT) [IKA/WERKE, Switzerland]

This equipment was used for the preparation of the emulsions as the homogenizer, which produces agitation at five different speed levels.

2.1.5.2 Heidolph [RZR 2020&21/40/41]

This is mixer is also used for homogenizing; this can operate with different speed levels.

2.1.5.3 Ostwald's viscometer

The viscometer used for analyzing the viscosity was Schott's type: 50905(285404022; 23/06).

2.2 Methods

2.2.1 Preparation of the emulsion

The emulsions were prepared by dispersing the oil phase (liquid paraffin) in to water with different amounts of surfactants under agitation of Ultra – Turrax, (UT) and were prepared with various time intervals. The emulsions were prepared using different compositions, under varied speed of agitation, time of agitation, processing etc. The emulsion studied can be demarcated broadly by the compositions involved, one is the concentrated emulsion containing equal proportions of the oil and aqueous phase with added surfactants and the other is the dilute emulsion containing lesser proportion of oil phase in large volume of aqueous phase. The study of the dilute emulsions grow to be critical especially in the analysis of emulsion stability by measuring droplet sizes demand dilution of the emulsion in some of the equipments used so direct approach was attempted by preparing dilute emulsions.

2.2.1.1 Preparation of the concentrated emulsion

Preparation with different agitation times:

The emulsions were prepared by dispersing 50ml of liquid paraffin in to 50 ml of water with 2ml of Polysorbate 80 and was agitated using Ultra-Turrax (UT) at a speed of 11500 rpm for 10, 20, 30, 60, 120, 180, 300 and 360 seconds respectively, the resulting emulsions are all different as they differ by the agitation speed levels. The following Table 4 name the emulsions prepared.

Table 4. Formulations presented at different agitation times

Formulation	Agitation time (s)
1	10
2	20
3	30
4	60
5	120
6	180
7	300
8	360

Preparation with different speed levels:

Also, emulsions were prepared by using different speed levels of the Ultra-Turrax ranging from 7400 rpm up to 24500 rpm. The duration of agitation was fixed to 120 seconds, the composition was kept constant and same procedure of dispersing the oil phase (liquid paraffin) of same ratio 1:1 was carried out, 50 ml of liquid paraffin dispersed to 50ml of water containing 2ml of Polysorbate 80. Table 5 lists the emulsions prepared. Table 6 represents the formulations prepared in the same way but the agitation duration was fixed to 10 seconds.

Table 5. Formulations containing different agitation speeds agitation duration 120 seconds

Formulation	Speed (rpm)
9	7400
10	11060
11	15500
12	20500
13	24500

Table 6. Different speed levels at agitation duration of 10 seconds

Formulation	Speed (rpm)
14	7400
15	11060
16	15500
17	20500
18	24500

Preparation with different surfactant composition

The emulsions prepared differ by the manufacturing process; the same procedure described under the section 2.2.1.1 was followed to prepare another set of preparation based on varying surfactant concentration, the same ratio was maintained between the oil phase and the aqueous phase and only the Polysorbate 80 concentration was kept varying corresponding to 2ml, 5ml, 10ml, and 20ml. The duration of agitation was carried out for 360, 600, 900 seconds for each surfactant concentration and the speed was fixed for 11500rpm. An effort to study the surfactant activity on different agitation levels was made. Table 7 indicates the corresponding formulations.

Table 7. Formulations with different surfactant volumes

Formulation	Liquid paraffin (ml)	Polysorbate 80 (ml)	Water (ml)
19	50	2	50
20	50	5	50
21	50	10	50
22	50	20	50

Preparation with different oil composition

Emulsions containing different oil volumes were prepared by dispersing varied volumes of oil comprising 40, 50, 60 and 70 ml with the constant volume of surfactant (Polysorbate

80) (20 ml). The agitation was carried out for 360 seconds and the speed was kept constant to 11,500 rpm and Table 8 represents the formulations.

Table 8. Formulations with varied oil phase volumes

Formulation	Liquid paraffin (ml)	Polysorbate 80 (ml)	Water (ml)
23	40	20	50
24	50	20	50
25	60	20	50
26	70	20	50

2.2.1.2 Preparation of dilute emulsions

The preparations of dilute emulsions involve reduction of the oil phase and enhancement of the aqueous phase as the dilution has to be made by increasing the continuous phase. 0.2ml of the oil phase (liquid paraffin) is dispersed in to water the continuous phase with different amounts of surfactants like Polysorbate 80 and sorbitan monolaurate using the agitation by Ultra turrax and is prepared with various time intervals. The emulsion is made up to 1000 ml with the continuous phase. The speed of the Ultra Turrax was kept constant to 11500rpm. Table 9 lists the corresponding formulations as d1, d2, d3, d4, d5 (d-dilute emulsions). Table 10 lists a formulation containing different volume of liquid paraffin.

Processing of the emulsion

a. Discontinuous processing.

The formulation (d1) was considered; the mixture of 0.2ml of liquid paraffin, 0.5 ml of Polysorbate 80 in 1000ml of water was agitated using the Ultra-Turrax with the speed of 11500 rpm for 1 min , the same system was run for additional times making up different processing times (2,4,6,8,10,15 minutes).

b. Continuous processing

The formulation (d1) was employed; the same mixture was agitated using Ultra-Turrax at 11500 rpm. The Ultra-Turrax agitation was carried non stop for 6, 10 and 15 mins respectively

Preparation with different type of stirrer

A different type of stirrer called Heidolph stirrer was also used to prepare the emulsion. The same procedure was followed as 0.2ml of liquid paraffin was dispersed in to water with 0.5 ml of Polysorbate 80 but here the Heidolph stirrer was used for agitation and it was for 900 seconds. Table 11 represents the formulation as d1h, d2h and d3h (d – dilute emulsion with Heidolph stirring).

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Table 9. Formulations with decreased volume of the oil phase and surfactant volume

Formulation	Polysorbate 80 (ml)	Sorbitan monolaurate (ml)
d1	0.5	-
d2	-	0.1
d3	0.1	-
d4	-	0.01
d5	-	0.05

Table 10. Formulation containing different volume of Oil phase

Formulation	Liquid paraffin (ml)	Polysorbate 80 (ml)
d6	0.5	0.5

Table 11. Formulation using Heidolph stirrer

Formulation	Speed used (rpm)	Polysorbate 80 (ml)
d1h	196	0.5
d2h	500	0.5
d3h	1425	0.5

2.2.2 Particle size Analysis

2.2.2.1 By Phase Doppler Anemometry:

The prepared emulsions were analyzed using the Dantec system, Germany. The PDA system consists of a laser (typically a continuous wave argon-ion-laser), fiber optics, and frequency shifter, transmitting and receiving optics, signal processor, traversing system and a computer to control the measurement and save the data. The size measurement of the particles is based on the optical detectors; the size measurement is based on the phase difference between the signals received by the two detectors. Coherent (Innova 70 series) argon-ion laser of 3W was used.

A glass tank was set up which was filled with water and the sample measuring cell was placed within as the path of the light would not be affected, the sample cell was connected to a funnel to facilitate the flow of the emulsions in to the cell from above (Figure 35). The measuring cell was selected with typical size taking in to account various parameters in order to minimize unnecessary lens effect promoted by the walls.

The emulsions were prepared and diluted accordingly coping with the possibility of the droplets to allow the light to pass through, and the prepared emulsions were passed through the funnel in to the cell at a constant velocity.

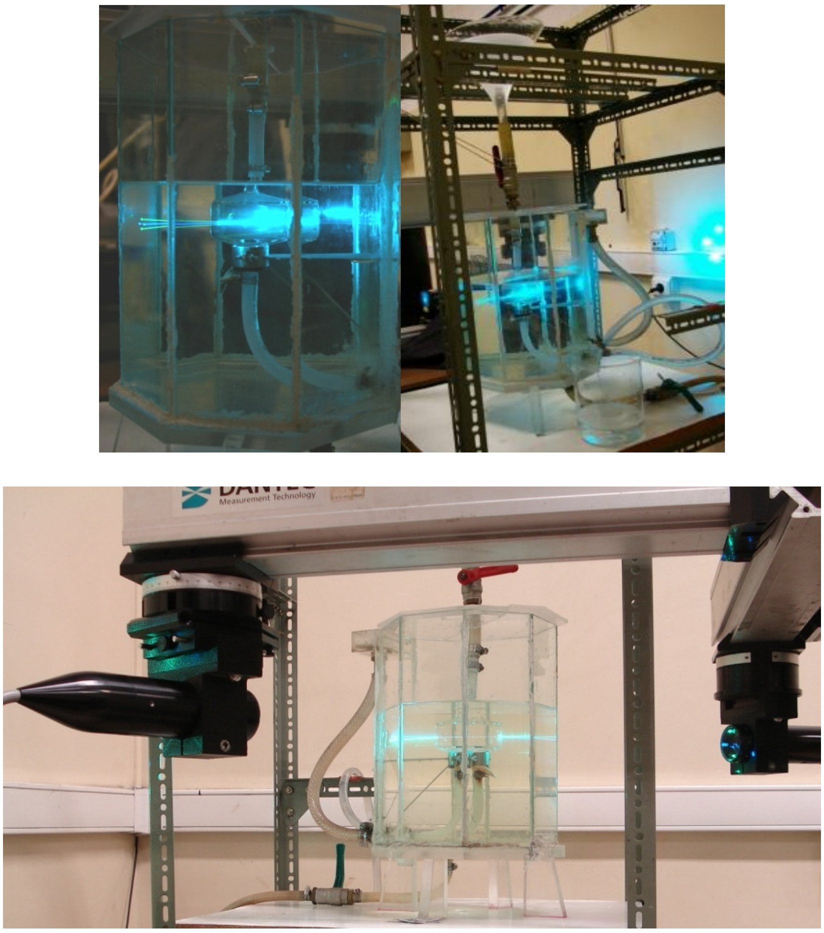


Figure 35. Photo of the measurement set up of the PDA system; The picture in the upper left shows the scattering from the emulsion droplets; picture in the upper right shows the full view of the set up, the bottom picture shows the position of the detectors

As the emulsion prepared flows through the funnel and reaches the sample cell where the measuring volume created by the two incident laser beams exist. The flow of the droplets in the emulsion scatters the light beam and the scattering was detected by the detectors. The transmitted waves interfere in space and create a beat signal with a frequency which is proportional to the velocity of the particle. The detectors receive this signal with different phases. The phase shift between theses two signals are proportional to the diameter of the particle. The arrangement can be further extended by directly measuring the particle diameter during the process of emulsification, the Ultra Turrax can be fitted above the funnel where the oil phase and the aqueous phase can be emulsified with added surfactant and when exists the flow of the on time formed emulsion to the sample cell, automatically the detectors receive the signals from the scattering by the emulsion droplet and thus the system can be well studied which contribute to the possibility of optimizing the emulsion.

2.2.2.2 Photon Correlation Spectroscopy

The average droplet size distributions were determined by photon correlation spectroscopy using Malvern zeta sizer 1000HSa. The sample was prepared by diluting the emulsion with the dimineralized water as the analysis can only be carried out with dilution. The cuvette was then introduced in to the instrument for analysis. The instrument consists of monochromatic coherent He-Ne laser with a fixed wavelength of 633 nm and this is used as the light source which converges to a minimum size in the sample by the use of focusing lens. (Manufacturer's web page) The scattered light is detected by the photomultiplier. Fluctuations in the intensity of the scattered light are converted in to electrical pulses which are fed in to a correlator. This generates the autocorrelation function which is passed to a computer where appropriate data analysis is done. This instrument cannot analyze very concentrated samples. The dilutions were made accordingly to meet with the equipments requirement and were analyzed. The droplet size was described by the cumulant mean (z-average) diameter.

2.2.2.3 By Laser diffraction

The Coulter LS 130 was used to determine size of the droplets produced in the emulsion. Coulter LS contains a fluid module of about 1.7 liters where the prepared emulsion (sample) was dispersed and the volume was recorded. The laser beam light up the dispersed sample, the polarized light beam illuminates the sample and scatter light in patterns determined by their sizes. This instrument uses the diffraction of Laser light by particles as the main source of information about the particle size.

The Laser power was set to be 5mw and the operating power of 4mw, the wavelength range was 750nm. This contains a PIDS lamp type of Tungsten-halogen.

The diffraction pattern were measured by the 126 detectors placed at angles to approximately 35 degrees from the optical axis, the relative amplitude of each diffraction pattern is used to measure the relative volume of the spherical particles of that size. This computation is based on the Mie model of light scattering by particles. The average mean diameter of the droplets by volume and mean diameter by number were obtained.

2.2.2.4 Microscopy:

The Microscopical observations were performed using an Optical microscope (Olympus CX40). Photo micrographs were generated using a digital camera Olympus U CMAD3 (Japan). The emulsions were diluted using the Milli-Q (Millipore Corporation) water. A drop of the diluted emulsion was carefully taken on a glass slide and covered with a cover slip before being observed through the microscope. The measurements of the size of the droplets were performed using the Cell^D imaging software (Olympus)

Chapter 3 RESULTS AND DISCUSSION

Considering the equipments used for the measurement of the droplet size distribution, some of the problems were encountered with the Coulter counter where certain quandaries with the software of the equipment occurred. Under the method, photon correlation spectroscopy (PCS), Malvern system utilized for the analysis has a particle size range that could be measured. The equipment is meant for the measurement size range up to 1 μm, Malvern evaluates the droplet diameter by running the analysis for 3 cycles and the cycles contains 10 runs each. Then more accurate value is taken as the result. It was seen through that the counts were consistent. During the measurement, the counts did not show any decrease on time meaning that no precipitation or instability was experienced by the emulsion. The equipment was managed properly for measuring the droplet diameter, all the settings was right, sample concentration utilized was appropriate for the measurement and no inconsistency was encountered during the measurement to obtain a valid result but still the measurements were done in the marginal upper limit of the equipment, the results of the PCS showed lesser mean droplet diameter ranges when compared with other methods as the equipment cuts out the higher mean values considering them as polydisperse even though the emulsion originally may not be polydisperse. All the equipments utilized for the analysis suffer the difficulty of dilution. The microscopy also required dilution. Each equipment demands dilution to different scales and that the dilution was made in accordance with respective requirement of the instrument and the comparison between the measurements exhibit difficulty. These equipments are being employed in many industries and laboratories currently for analysis although they provide relevant data, the mentioned difficulties stand as the obstacle. As discussed previously under the section of measurement techniques online monitoring techniques like back scattering techniques (FBRM, Turbiscan) reduce many of these complexities as no dilution is required and direct measurement can be done.

3.1 Effect of agitation time

In the following section the effect of the agitation time on the stability of the emulsion is discussed. Experiments were run according to (Table 4).

The emulsions formed were all different due to different agitation time. It was observed that by increasing the agitation time the consistency of the emulsion increases. The insufficient agitation time did not disperse evenly the oil phase in the aqueous phase. The agitation produced by the homogenizer is mainly the mechanical force which helps to disperse the oil in aqueous phase. In addition the formed droplets might undergo coalescence to yield larger droplets due to inadequate covering of the surfactant on the droplets. This clearly explains the different size droplets formed during short agitation time resulting in non-homogenous emulsion formation. The emulsion is required to spend sufficient time in the agitation zone or the emulsification zone in order to get the droplets completely disrupted. Less disruption of droplets occurs when the emulsion does not spend sufficient time [23].

The agitation time in the process of emulsification present as an important criteria on stability study of an emulsion. Insufficient agitation time resulted in non-homogeneous droplets in the aqueous phase. Decrease in droplet size distribution was observed when there was increase in agitation time. Figure 36 shows the decrease in droplet size distribution along the increase in agitation time. It is evident from the graphical representation that a pattern exists demonstrating a slight upward lift in droplet diameter which then falls consistently.

Table 12. Effect of Agitation time on the mean diameters of drops measured by different equipments

Formulation	Time (s)	Variation in Mean diameters (μm) between methods due to different dilutions				
		PDA		PCS*	L.D	
		mean	s.d	mean	mean	s.d
1	10	-	-	-	-	
2	20	8.70	4.65	2.40	1.26	0.76
3	30	9.60	5.53	4.15	1.14	0.91
4	60	10.0	5.66	3.72	2.18	1.20
5	120	9.00	4.73	3.35	1.50	1.19
6	180	8.10	5.50	2.80	0.29	0.11
7	300	8.40	5.34	2.30	0.16	0.06
8	360	7.40	4.60	2.30	0.16	0.10
Amount of Polysorbate 80 (moles)		3.194×10^{-5}		3.194×10^{-6}	3.994×10^{-4}	
Initial amount of Polysorbate 80		1.63×10^{-3} moles				

* For PCS analysis the standard deviation was not included as the polydispersity for the analysis exhibited the same value for all the experiments

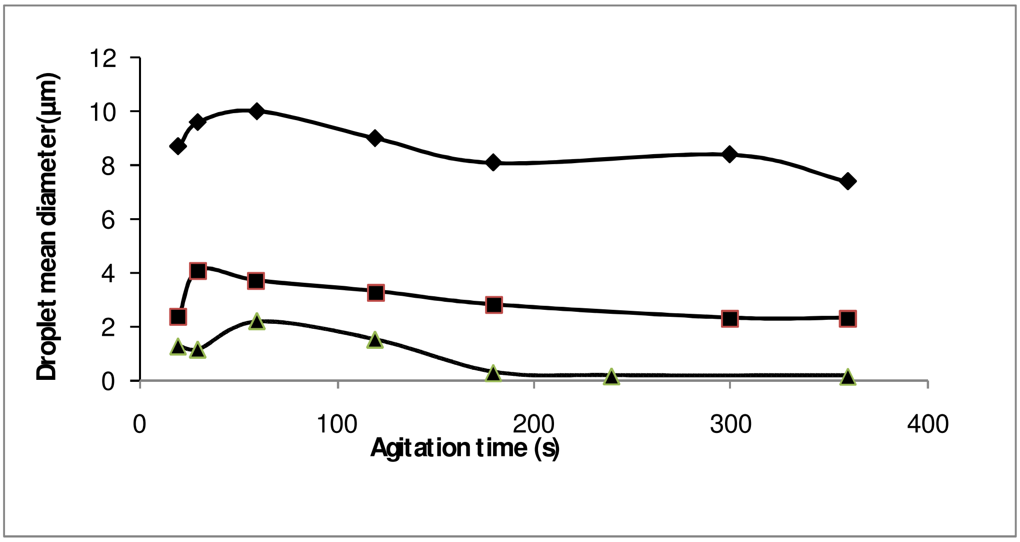


Figure 36. Effect of agitation time on the size of the droplets; (♦)PDA (■)PCS (▲)LD

Here, emulsions corresponding to formulations 1 to 8 (Table 4) were employed. The graph depicts the effect of the agitation as the function of time. PDA analysis of the droplet sizes of the emulsions shows a decline in diameter along the increase in agitation time. PCS analysis go along the same decline with increase in agitation time, the Coulter measurements also indicate a decrease in size. A slight pattern is seen with all methods employed. A thin step up in droplet diameter and then fall in diameter is noticed.

Considering PDA graph, we could notice a slender rise in droplet diameter at the 20, 30 seconds (formulation 2 & formulation 3) and then the fall in the size. Standardized trend is seen with the PCS and LD analysis. This could be because of less contribution of agitation time. Less time in the agitation environment leads to insufficient covering up of the surfactant molecules around the droplet which in turn leads to coalescence. In all the three methods a pattern is noticed at the same time the droplet mean diameters are consistent and do not show any in consistency.

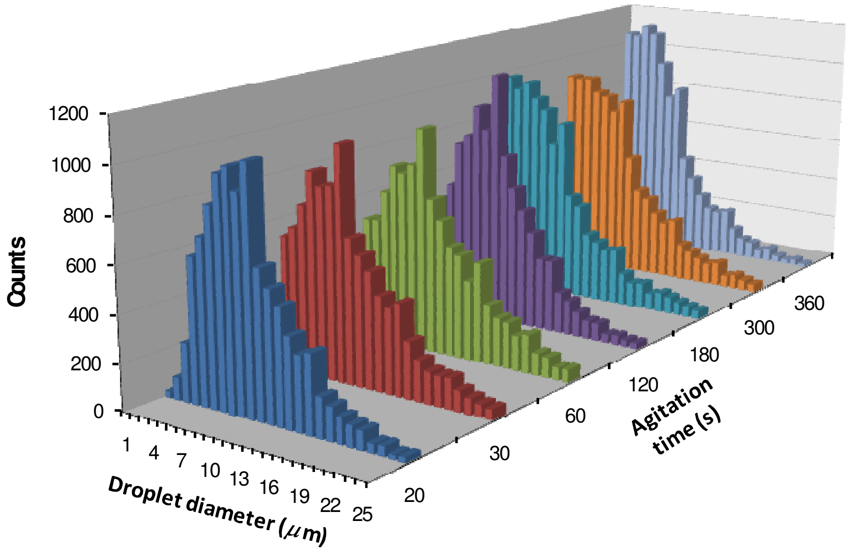


Figure 37. Comparison of mean distribution graphs of PDA analysis with different UltraTurrax agitation

The PDA analysis of the droplet size distribution is depicted in the Figure 37. It clearly shows that the droplet size distribution getting narrower as the agitation time increases. In the initial seconds of agitation the distribution seems to be wider with larger mean values. This decrease in droplet diameter along the increase in agitation time is in accordance with the results of Kawaguchi et al [56], were the size of the oil droplets were analyzed in the emulsions stabilized by the poly(oxyethylene) 10-lauryl ether (OE-10) with different agitation times it was further stated that, the volume fraction of oil higher than 0.7 is independent of the agitation time and droplets increases with the decrease of the corresponding surfactant volume.

3.2 Effect of the homogenizer speed

The shear forces generated in the turbulent zone within the gap between the spinning rotor and the stationary rotor is the main factor which determines the size of an emulsion droplet. A vacuum is generated by the rotor which is concentrically placed inside the stator; the rotor rotates and the generated vacuum draws the liquid in and out of assemble there by resulting in circulation. Two major forces can reduce the size of the dispersed droplet; the main force is the mechanical impingement against the wave due to high fluid acceleration. The other shear force takes place principally within the gap between the rotor and the stator. At high rotational speeds the flow would be highly turbulent [57].

The homogenization intensity and the residence time of the emulsion droplets in the shearing field are the primary parameters for controlling the emulsion droplet size. The turbulence created is much intensive as it was achieved by rapid flow circulation and higher shear force within the narrow gap between the rotor and the stator. Thus homogenization intensity plays a primary role in droplet size distribution. So, the influence of homogenization intensity on the droplet size distribution was studied. The emulsion was prepared using various homogenization intensity agitations for a particular period of time. It was observed that the emulsion droplet size decreases along the increase in the homogenization intensity. The emulsions were prepared using the intensities ranging from 7500 rpm to 24000 rpm and results of droplet size distribution appear to be decreasing along the increase in the intensities. In this analysis, PDA and Microscopy can be compared as such as the analysis are performed from the same formulations Table 5 but the PCS analysis was done using the formulations 14 to18 (Table 6). There exists a difference in agitation time with PCS analysis. The emulsion was agitated for 10 seconds instead of 120 seconds as in the other two methods. The results are still comparable. Figure 38 shows the decrease in droplet diameter against the increase in homogenizer speed. A pattern is met also in this figure a slight upward incline and then the fall in droplet diameter. The PCS analysis is depicted in the graph as the dotted lines in order to differentiate it from other analysis.

Table 13. Effect of homogenizer speed on the mean droplet diameter.

Formulation	Speed (rpm)	Variation in Mean diameters (μm) between methods due to different dilutions			
		PDA		Microscopy	
		mean	s.d	mean	s.d
9	7400	-	-	209.78	21.00
10	11060	7.40	3.85	226.72	25.21
11	15500	7.90	6.102	160.21	10.08
12	20500	6.30	5.099	166.75	17.64
13	24500	6.00	4.104	130.75	31.09
Amount of Polysorbate 80 (moles)		$3.194x10^{-5}$		$1.591x10^{-6}$	
Amount of Polysorbate 80 (moles)- Initial		$1.63x10^{-3}$			

Table 14. Effect of homogenizer speed on the mean droplet diameter by PCS analysis

Formulation	Speed (rpm)	Mean diameter (μm)
		PCS
14	7400	1.18
15	11060	2.87
16	15500	1.88
17	20500	1.88
18	24500	1.50
Amount of Polysorbate 80 (moles)		$3.194x10^{-6}$
Amount of Polysorbate 80 (moles)- Initial		$1.63x10^{-3}$

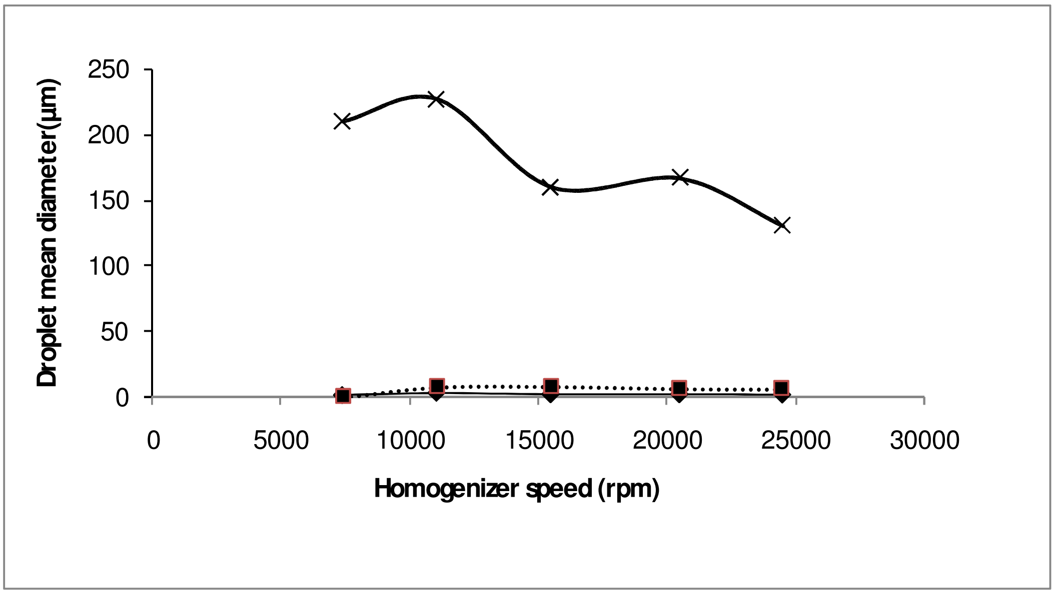


Figure 38. Effect of different homogenizer speed; (♦)PDA analysis, (··■··) PCS (**x**) Microscopy

Increasing the speed of the homogenizer decreases the droplet diameter. This effect is clearly visualized by the graphs.

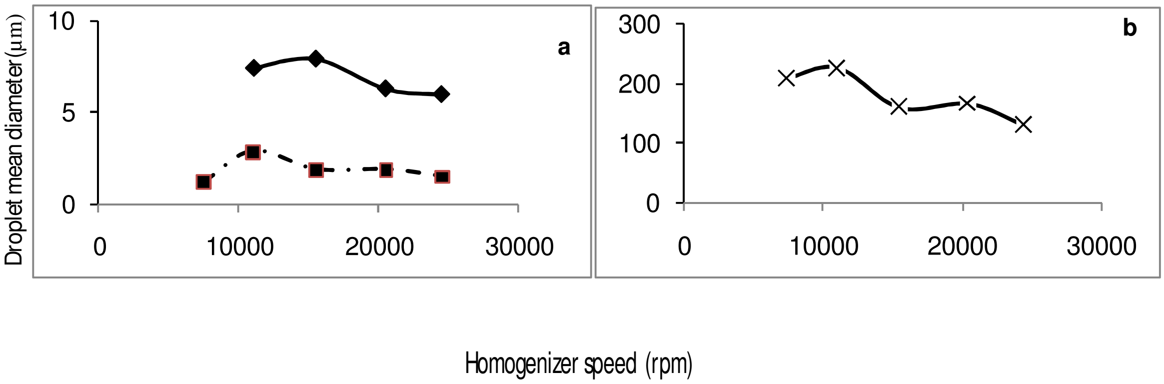


Figure 39. Graph depicting the homogenizer speed values (rpm) plotted against droplet mean diameter(µm); Left (♦ - PDA, ■ – PCS), Right (x - microscopy)

When we consider Figure 39 (a) a typical pattern is seen with the two methods (PDA and PCS) introducing an increase and then the stabilization. However there should a different effect with the PCS analysis as the study was done with less duration of agitation. The droplets are supposed be enlarged. On the contrary PCS analysis shows the lowest of the droplet diameters in this study of homogenizer efficiency. Microscopy analysis show bigger droplets which are far bigger than the other two methods even though the pattern remains the same. The droplets measure around 200 microns and PDA analysis represent droplets around 7 microns whereas PCS analysis shows droplets to 1 micron. The droplet distribution results from different methods show different values. Excluding these differences the results directly convey that that homogenizer effectively plays the role in reducing the droplet size. Increasing the efficiency of the homogenizer or increasing the agitation speed decreases the droplet diameter. The obtained results depicting the decrease in diameter with increase in agitation rate or agitation speed can be related to the results of Franco et al. [58]. Further the Mostaedi et al. [59], also quoted that drop size decreases with agitation speed where the agitation speed efficiency of the Hansen mixer- settler was studied.

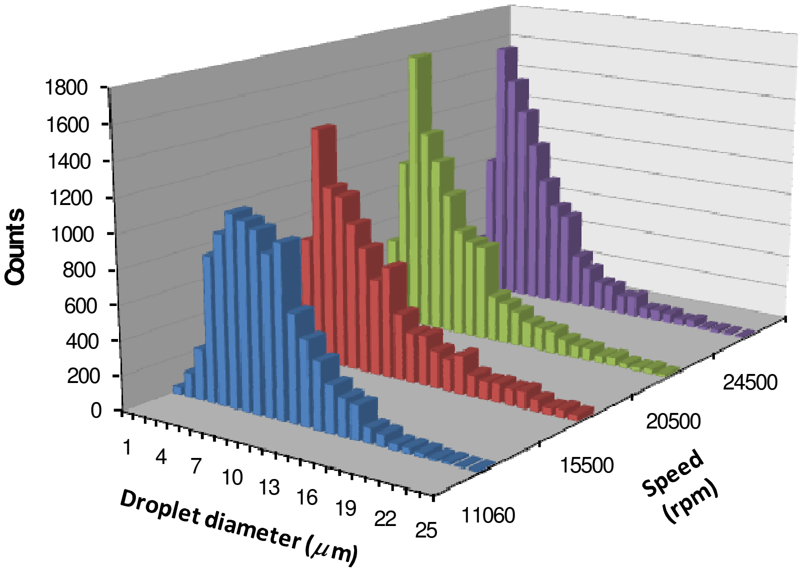


Figure 40. Comparison of mean distribution graphs of PDA analysis with different Speed of Ultra Turrax agitation

In Figure 40 the droplet distribution pattern clearly suggests that increasing the speed level drastically reduced the droplet diameter. A very sharp change in the mean distribution is noticed in each histogram. The mean distribution is getting narrower step by step along the increase in the speed level.

3.3 Effect of the surfactant concentration

An important influence of a surfactant is it increases the surface viscosity and the movements of the interface are greatly reduced thus showing down or masking coalescence. As surfactant concentration seemed to be an important criterion, the effect was studied.

The paraffin oil in all emulsions was kept constant at 50% volume and same was the water volume. The surfactant volume was varied from 2 ml, 5 ml, 10 ml and 20 ml. The droplet size distributions were calculated using phase Doppler anemometry (PDA), photon correlation spectroscopy (PCS), Laser diffraction method (LD) and Microscopy. The results were compared; it was observed that the droplet size distribution and the average diameters decrease with increase in the amount of surfactant. Addition of surfactant improves the stability of the emulsion.

The study of droplet size distribution (DSD) of the emulsions at different surfactant volumes were presented in the Figure 41. We could conclude that with the increase of the surfactant concentration, the droplet size becomes narrow and the initial average diameter of the droplets declines steady. This is mainly because surfactant concentration governs the total interfacial area and hence the average size of the emulsion droplets.

Table 15. Droplet mean diameters values for different surfactant volume

Formulation	Polysorbate (ml)	Agitation time (s)	Variation in Mean diameters (μm) between methods due to different dilutions				
			PDA		PCS	Microscopy	
			mean	s.d	mean	mean	s.d
19	2	360	10.01	4.15	3.09	266.90	76.47
		600	9.72	11.23	2.90	178.71	30.25
		900	9.22	4.83	2.44	136.85	12.60
20	5	360	6.30	4.20	2.89	15.42	0.21
		600	6.35	3.84	2.46	-	-
		900	5.20	3.34	2.31	-	-
21	10	360	5.51	3.30	2.28	4.59	0.75
		600	4.31	2.77	2.58	-	-
		900	4.30	2.70	2.27	-	-
22	20	360	5.56	3.31	1.88	2.33	0.66
		600	3.86	3.00	3.73	-	-
		900	3.60	2.32	3.57	-	-

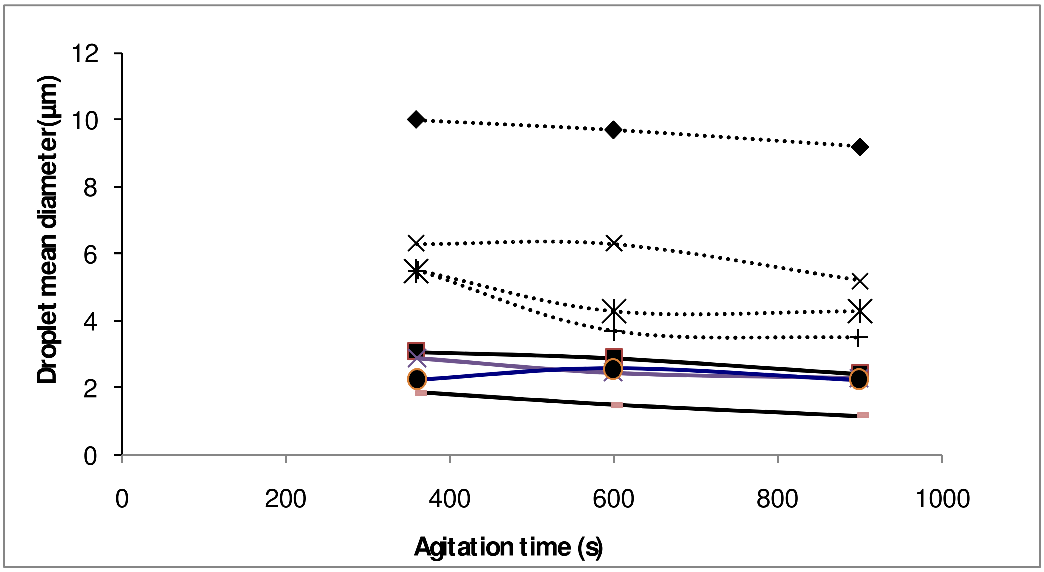


Figure 41. Effect of surfactant and agitation time on the droplet mean diameter; [PDA analysis ···♦··· (2ml) ···x·· (5ml) ····ж·(10ml), ···+·· (20ml)], [PCS analysis ■(2ml), -x-(5ml), -•-(10ml), -(20ml)], Formulations 19 - 22

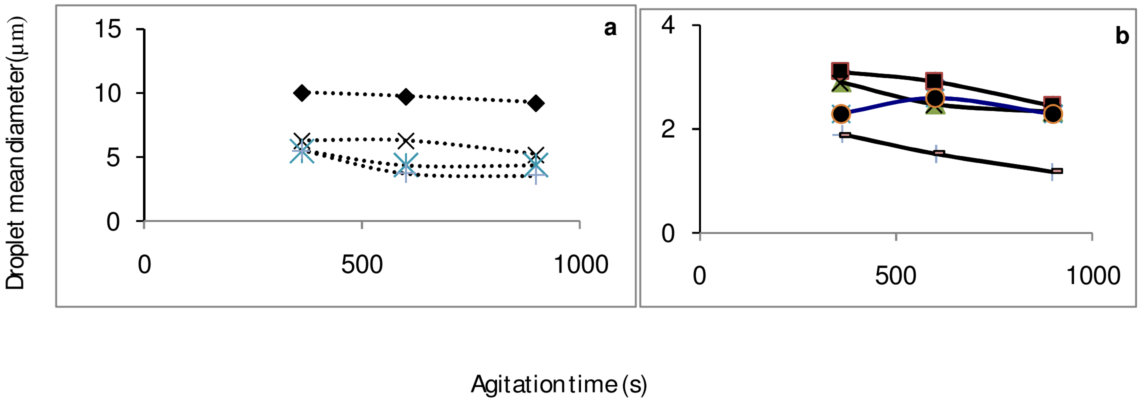


Figure 42. Seperate representation of PDA and PCS analysis. Graphs depicting the agitation time values (s) plotted against droplet mean diameter values (µm); (a) PDA analysis, (b) PCS analysis, Formulations 19- 22

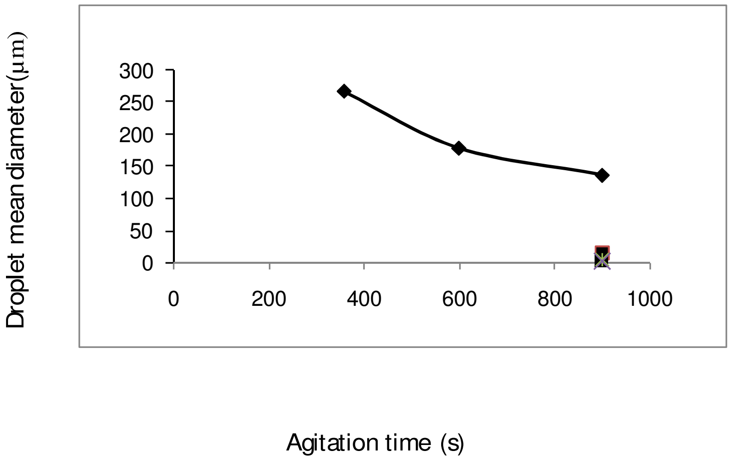


Figure 43. Graphs depicting Microscopy analysis where the agitation time values (s) plotted against droplet mean diameter values (µm) Formulations 19- 22. [◇] (2ml), [■] (5ml), [ж] (10ml), [✱] (20ml)

The graph Figure 41, clearly point toward a decrease in droplet diameter along the increase in surfactant volume. Both increase in agitation time and increase in surfactant concentration comparably reduce the mean diameter of the droplets.

Regarding Formulation (19) 2ml of the surfactant (Polysorbate 80) is taken in to account, in the Figure 42 with the PDA analysis (··♦··) shows a decline in diameter which is consistent, there is good descend the same effect was seen when analyzed by photon correlation spectroscopy (■) but here the diameter of the droplets are much smaller. The curve slides down in both the cases. When the formulations 20 and 21 are considered, the surfactant volume would be 5ml and 10 ml respectively, PDA analysis(···× ···)(··ж··), and PCS analysis (Ӿ), (•) also indicate the same trend but here the curve slightly descends and stabilizes a bit, this is relevant in both the analysis. The formulation 22 shows a fall in droplet diameter in the case of PCS (-–-) analysis and also with the PDA (···+··) (20ml). It can be visualized easily from the graph but at the same time not very big descend. The graphs suggest clearly that addition of surfactant produces smaller droplets. When the formulation 21 of the PCS analysis (-•-) was considered which consists of 10 ml of the added surfactant, a slight dissimilarity at 600 seconds agitation was observed.

Microscopy analysis also show the same pattern when the Formulation 19 (◊) (2ml of the surfactant) was considered, where it shows a consistent decrease in diameter along the agitation time and the volume of the decrease was found larger when compared with other analysis.

Table 16. Comparison of Mean values obtained with different surfactant volume at a agitation rate of 360 seconds

Formulation	Surfactant Volume (ml)	Variation in Mean diameters (μm) between methods due to different dilutions		
		PDA	PCS	Microscopy
19	2	10.0	3.09	266.90
20	5	6.3	2.89	15.41
21	10	5.5	2.28	4.59
22	20	5.5	1.88	2.33

Table 17. Comparison of Mean values obtained with different amounts of surfactant with an agitation rate of 360 seconds

Amount of Surfactant (moles) Initial	**Amount of Surfactant (moles)** PDA	**PDA**	**Amount of Surfactant (moles)** PCS	**PCS**	**Amount of Surfactant (moles)** Microscopy	**Microscopy**
1.63×10^{-3}	3.194×10^{-5}	10.0	3.194×10^{-6}	3.09	1.591×10^{-6}	266.90
4.08×10^{-3}	7.777×10^{-5}	6.3	1.553×10^{-6}	2.89	1.599×10^{-4}	15.41
8.16×10^{-3}	1.484×10^{-4}	5.5	1.484×10^{-6}	2.28	1.600×10^{-4}	4.59
1.63×10^{-2}	2.720×10^{-4}	5.5	2.267×10^{-5}	1.88	1.333×10^{-3}	2.33

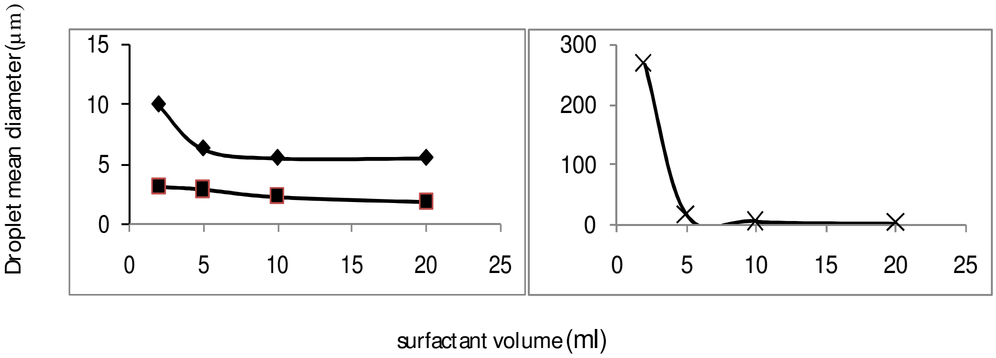


Figure 44. Graphical representation showing the surfactant volume (ml) plotted against droplet mean diameter values (µm).Left (PDA and PCS analysis, (♦ – PDA, ■ – PCS). Right (x - microscopy analysis)

The formulations 19-22 (Table 16) were referred here to plot this graph which compares PDA and PCS measurements which noticeably indicates that increase in surfactant volume decreases the droplet diameter of the emulsion.

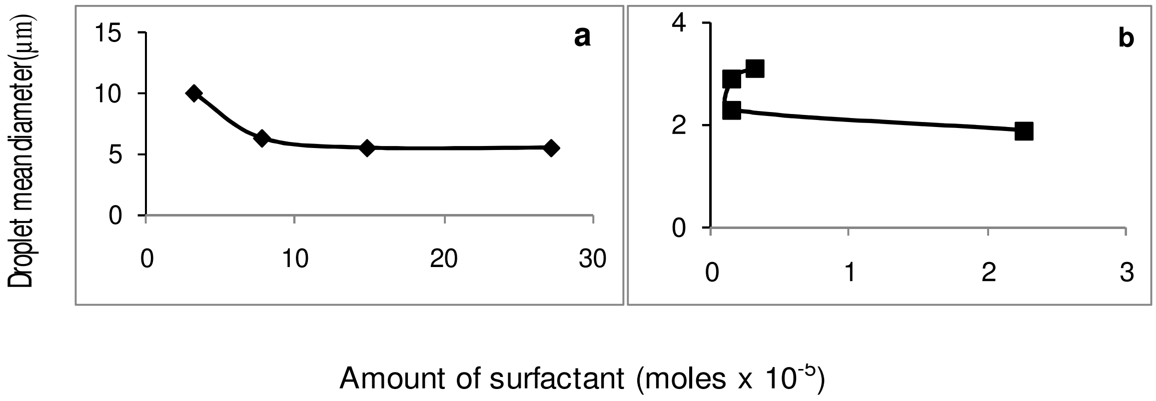


Figure 45. Graphical representation showing the amount of surfactant (moles x 10^{-5}) plotted against droplet mean diameter values (µm). Left (a) (♦ – PDA analysis), Right (b) (■ – PCS analysis)

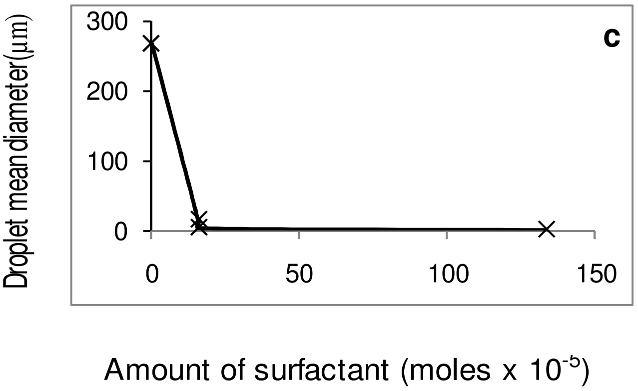


Figure 46. Graphical representation showing the amount of surfactant (moles x 10^{-5}) plotted against droplet mean diameter values (µm) in (c) Microscopy analysis (x)

The amount of surfactant in moles against the droplet diameter presented in the Figure 45 and Figure 46 depict the relation with the Figure 44 as the figures show an overlaying pattern. The analysis of the PCS demonstrates the effect of surfactant by showing reduction in droplet diameter but the analysis for formulation 21 shows a slight dissimilarity at 600 seconds agitation showing an increase in diameter. PDA analysis also illustrates the reduction in droplet diameter, with formulation 21 and formulation 22 (Table 15) the emulsion droplet changes stabilize. Considering the microscopy analysis, the results indicate a big difference in droplet diameter (Figure 46). PCS shows a consistent decrease in diameter and so do PDA, with microscopy, the droplet diameter of the formulation 19 (2ml of surfactant) (Figure 44) was to the tune of 266.90 µm then with the addition of surfactant it shows a straight forward fall in the droplet diameter, the droplets shrink down to about 15 μm and then on further addition of surfactant declines to the tune of 2 μm. The microscopy results represent a wide range of distinction in droplet size up on the surfactant addition.

Surfactant addition reduces the coalescence of disrupted droplets [23] which can be achieved by increasing the surfactant concentration or by a choice of a fast adsorbing surfactant. The guidance of the flow must be skillfully designed in a system to lead reduction of coalescence until the surfaces are completely covered by the surfactant molecules.

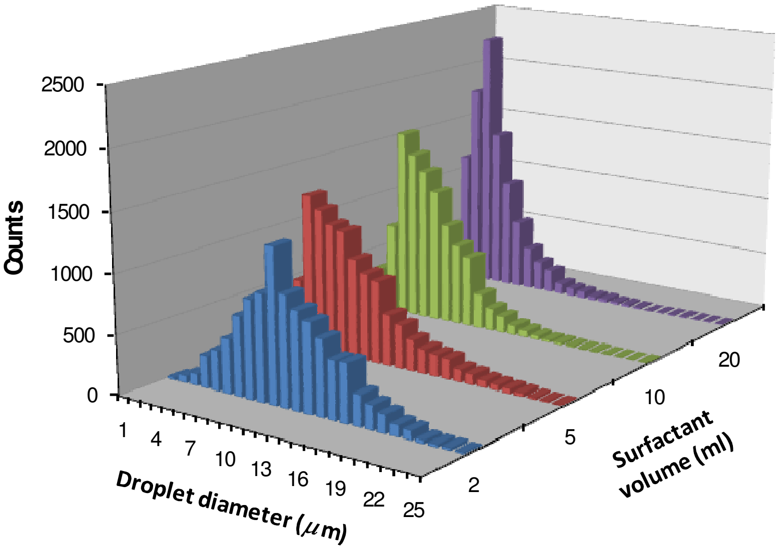


Figure 47. Comparison of mean distribution graphs of PDA analysis with different surfactant volume

Figure 47 represents the histograms of different surfactant volume. This explains clearly the change in distribution. A stepwise improvement in the pattern, at 2 ml of surfactant volume, the mean distribution is wide and from 5ml of surfactant the distribution is getting narrower with 10 ml it is very clear the effects of surfactant addition, the distribution is narrow and mean diameter gets reduced.

This logical decrease in droplet diameter along the addition of surfactant shows good agreement with the results of Roland et al. [24], in systematic characterization of oil in water emulsions in formulation design. The results can be compared with the ones of Liu et al. [22], where it was concluded that the droplet size distribution becomes narrow and the average radius of the droplet declines with the increase in surfactant concentration. Lizarraga et al. [60], showed that the droplet diameter decreased constantly when Ultra turrax (UT) was used and with the addition of (WPC) whey protein concentration as the sole emulsifier.

3.4 Effect of oil concentration

The larger the droplets of the dispersed of the dispersed phase (internal phase) of an emulsion are, the greater is the tendency to coalesce and further increase the droplet size and destabilize the emulsion and enhance the phase seperation. The relative proportion of oil in water also afffect the emulsion stability. Lower the oil content in the emulsion, the droplets have lesser chance of colliding with each other and coalescing than the emulsion with a larger number of dispersed oil droplets. Increase in oil droplets increases the droplet diameter.

Formulations 23-26 containing different proportions of oil concentration were analysed by PDA, PCS and microscopy.

Table18. Mean values obtained with different oil volume

Formulation	Oil volume (ml)	Variation in Mean diameters (μm) between methods due to different dilutions					
		PDA		PCS		Microscopy	
		mean	s.d	mean	s.d	mean	s.d
23	40	4.50	2.87	1.92	0.707	32.98	12.12
24	50	5.50	3.30	1.88	1.00	-	
25	60	7.50	4.67	2.22	0.273	38.88	5.04
26	70	9.90	4.32	2.71	0.22	47.52	5.12

Table 19. Amount of oil (moles) and mean values

Amount of oil (moles) Initial	Amount of oil (moles) PDA	PDA	Amount of oil (moles) PCS	PCS	Amount of oil (moles) Microscopy	Microscopy
1.74×10^{-1}	3.570×10^{-5}	4.50	2.968×10^{-5}	1.92	2.131×10^{-3}	32.98
2.17×10^{-1}	3.520×10^{-5}	5.50	3.625×10^{-5}	1.88	-	-
2.55×10^{-1}	3.890×10^{-5}	7.50	3.922×10^{-5}	2.22	2.182×10^{-3}	38.88
2.97×10^{-1}	4.210×10^{-5}	9.90	4.250×10^{-5}	2.71	2.432×10^{-3}	47.52

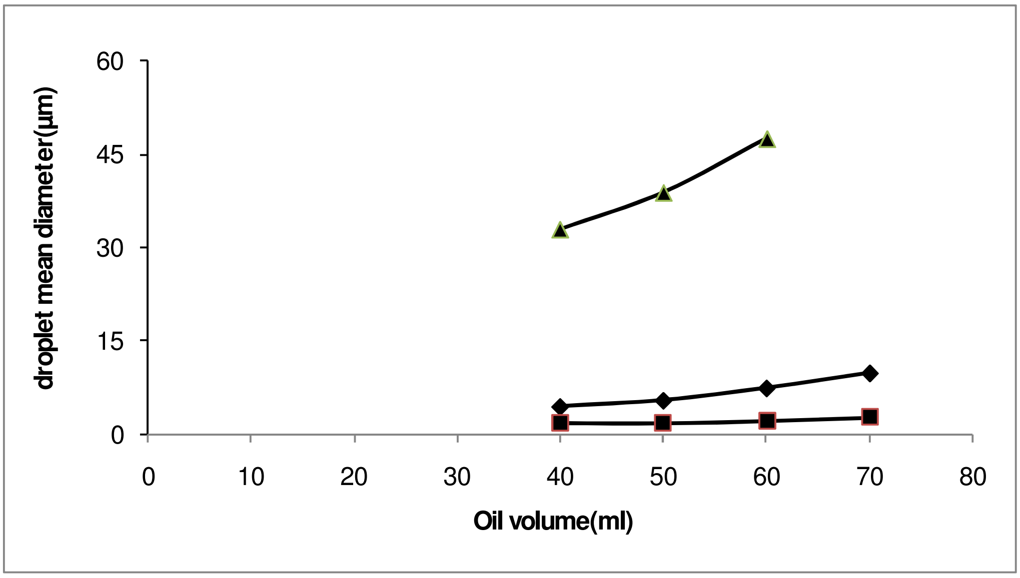
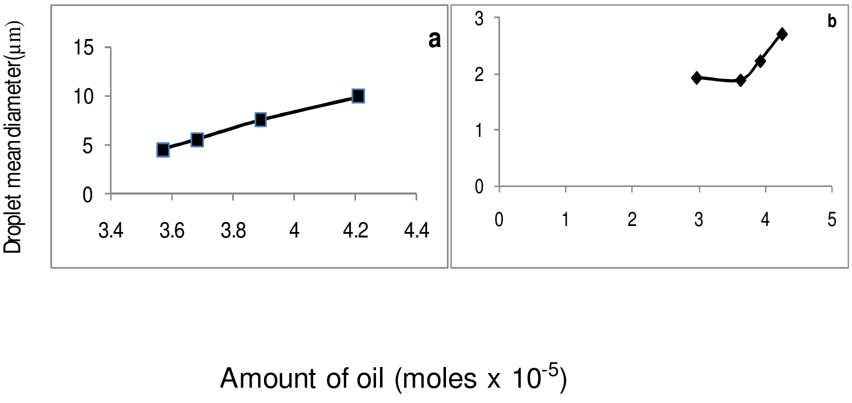


Figure 48. Graphical illustration of the effect of oil volume (ml) to the droplet mean diameter(μm), (♦-PDA analysis) (■– PCS analysis) (▲- microscopy)



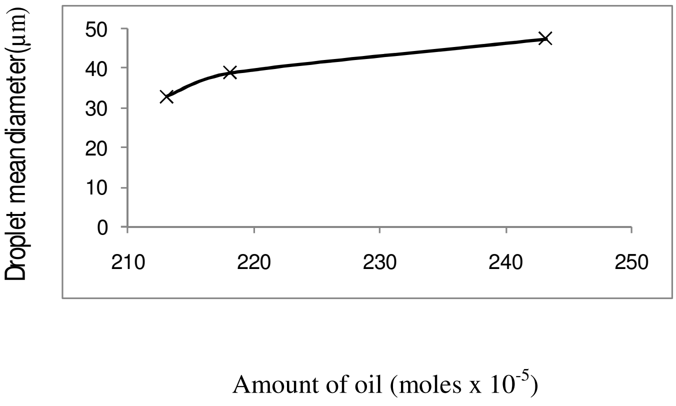


Figure 49. Graphical representation showing the amount of oil (moles x 10-5) plotted against droplet mean diameter values (µm) (a)PDA analysis (b) PCS analysis (c) Microscopy analysis

The PDA analysis on the effect of oil concentration illustrate that the droplets increase in diameter on oil addition. There exists a consistent increase in the diameter, the droplets grow to a tune of 2µm consistently explaining that increase in oil concentration causes less availability of the surfactant molecules to adsorb as the surfactant volume remains constant. PCS analysis indicates the same pattern but the rise is not very remarkable, ascend in the droplet diameter is to the tune of 0.3 to 0.4 µm. The microscopy approach illustrates an increase in diameter to the tune of 6 µm and the rise is not stopped

All the three methods showed an increase in droplet diameter along the addition of the oil. The trend is increasing which clarifies that enhancing oil concentration increases droplet diameter. These results showing an increase in droplet size on addition of the oil volume is in good agreement with the results of Juan C. López et al. [61]. Lobo et al. [62] suggests that increased oil phase loading always increases the amount of coalescence and increase in droplet size was observed.

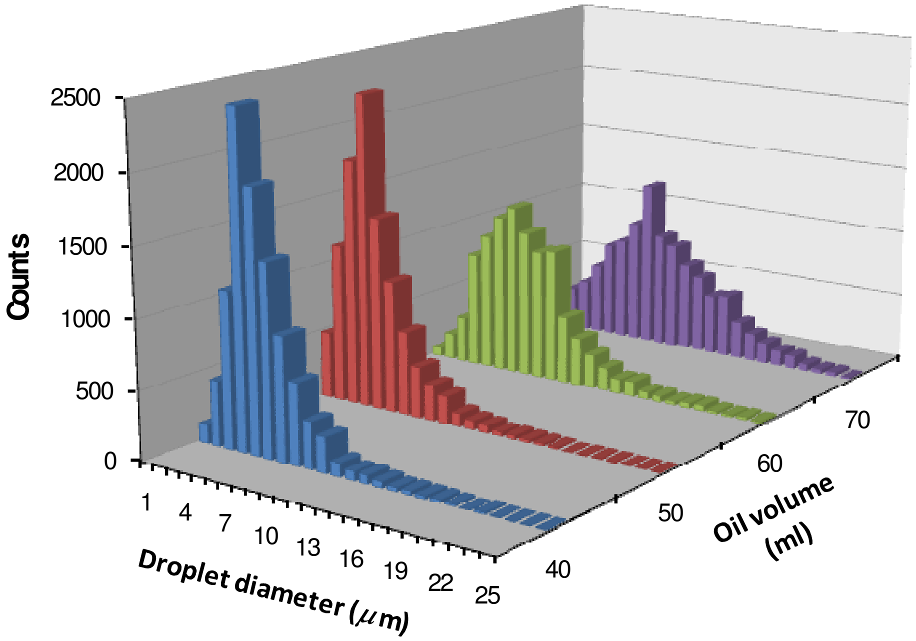


Figure 50. Comparison of mean distribution graphs of PDA analysis with different oil volume

Figure 50 shows a elucidates the increasing trend of the droplet mean distribution, the narrow distribution gets wider step by step in accordance with the addition of the oil volume.

3.5 Effect of ageing

Once the emulsification process was completed, the droplet size distribution was measured as a function of ageing. The ageing effect on the emulsions was studied. The formulation (8) was taken and the droplet size measurement was done after 5 minutes, 30 minutes, 1 hour and 2 hours. A steep increase in the droplet diameter was observed. The effect of ageing is an important criterion to understand the kinetics of emulsification; which serves us a clear understanding about the stability factor of the emulsion. Along the time, the droplet diameter increases.

The factors causing the increase in diameter and contributing the ageing effect are clearly represented by the following diagram.

Table 20. Mean droplet diameter of the aged formulation [8].

Ageing (s)	Variation in Mean diameters (µm) due to different dilutions						
	PDA		PCS	LD		Microscopy	
	mean	s.d	mean	mean	s.d	mean	s.d
30	-	-	-	0.16	-	-	-
900	-	-	--	-	-	-	-
1800	-		-	0.17	0.174	-	-
3600	-	-	-	0.17	0.135	122.00	2.52
7200	11.10	5.30	3.19	0.17	0.173	127.00	28.80
10800	11.50	5.11	3.65	1.31	0.786	285.00	78.99
13200	13.80	6.70	5.27	1.30	0.931	367.00	66.38
Amount of Polysorbate 80 (moles)	3.19×10^{-5}		3.194×10^{-6}	3.99×10^{-3}		1.59×10^{-6}	

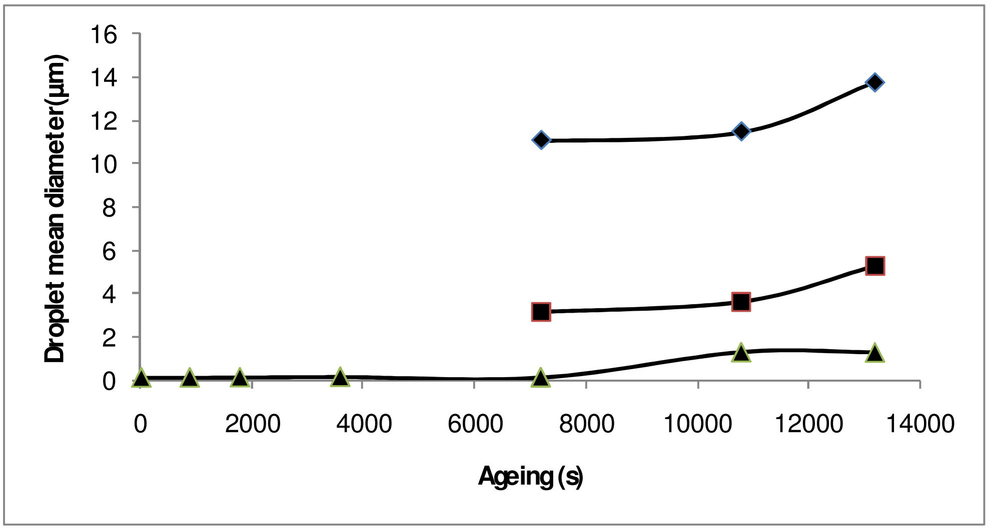


Figure 51. Graphical representation of the ageing process for the formulation [8]; ♦ PDA analysis, ■ PCS analysis, ▲ LD analysis

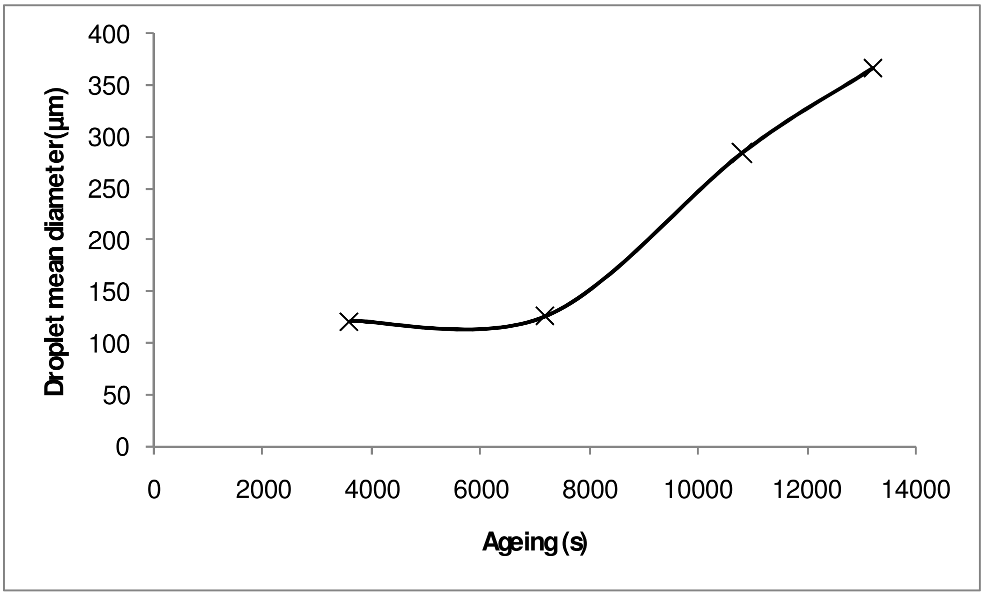


Figure 52 Graphical representation of the ageing process for the formulation [8], **x** LD Microscopy analysis

It shows increase in droplet diameter along the ageing of the emulsion. The LD analysis plateaus out at the end whereas PCS and PDA analysis and microscopy analysis represents a rapid raise in diameter on ageing of the emulsion and no decline after.

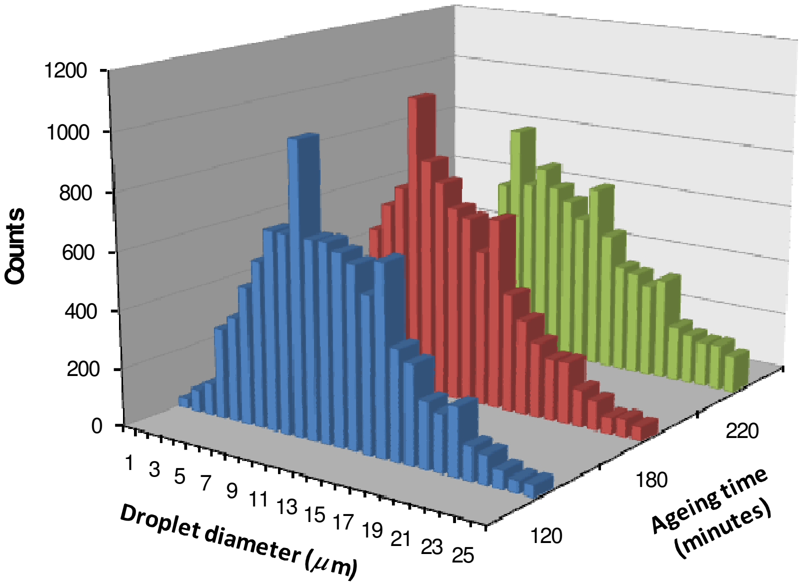


Figure 53. Comparison of mean distribution graphs of PDA analysis on ageing

On ageing the droplets coalesce and tend to grow bigger showing a prominent change in various features leading to phase separation at the final stage. Figure 53 depicts the change in the mean distribution along the ageing. The distribution gets wider along the ageing. The swelling of the emulsion droplets or increase in size of the droplets are in good agreement with the results of [63] where it was stated that the optical properties of emulsions were influenced by Ostwald ripening because the transport of oil molecules from smaller to larger droplets caused a gradual increase in the mean droplet size.

3.6 Dilute emulsion

3.6.1 Emulsion processing

As described in the methods section 2.2.1.2, the prepared and processed emulsions were measured using PDA. The two types of processing were carried out as explained under the same section, the discontinuous and the continuous processing. PDA analysis on the discontinuous processing and continuous agitation was logical as the equipment evaluate the changes that take place during the procedure. The discontinuous processing should be very much different from the continuous as the changes that take place in both the cases may be very different. The emulsion processing analysis was performed with PDA and only continuous processing was done with LD method. Malvern system of the PCS method did not support this study because of the high dilution.

The results from the discontinuous processing show a decrease n droplet diameter however not a big difference was observed between each measurements still an incline or a slope was observed Figure 54 showing a decrease in droplet diameter. Continuous processing did show a curve pattern for the reason that the 600 second agitation value showed a rise in diameter instead of declining.

Table 21. Discontinuous processing by PDA

Agitation time (s)	(μm)	
	mean	**s.d**
60	5.847	3.16
120	5.791	3.01
240	5.018	3.22
360	4.949	3.11
600	4.171	2.70
900	4.290	3.16

Formulation d1, Amount of Polysorbate 80 (moles): $4.08x10^{-4}$

Table 22. Continuous processing by PDA

Agitation time (s)	(μm)	
	mean	s.d
360	5.248	3.09
600	5.67	4.78
900	5.004	4.09

Formulation-d1, Amount of Polysorbate 80 (moles): $4.08x10^{-4}$

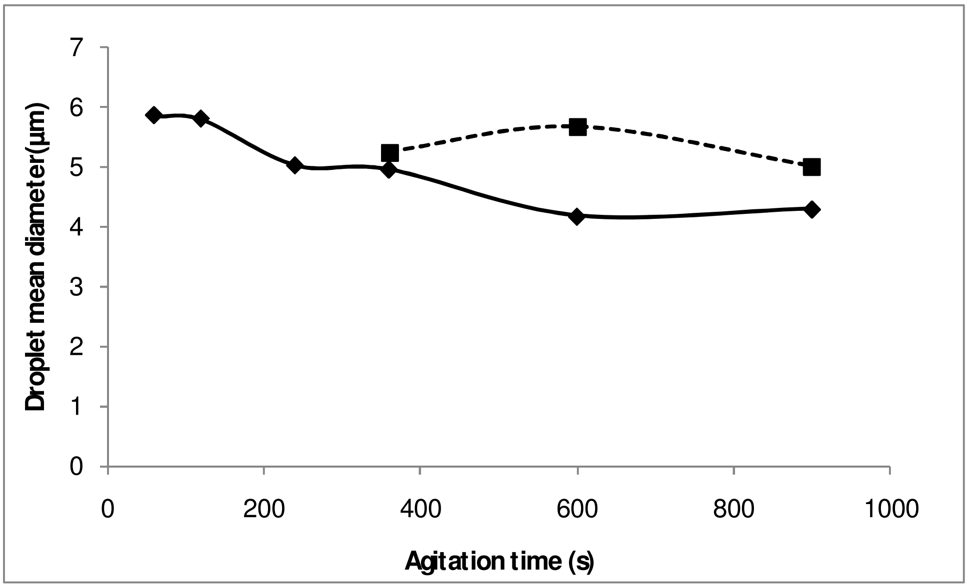


Figure 54. Emulsion processing – Graphical representation; (■) – Continuous processing, (♦) – discontinuous processing

The graph explains the effect of time for the processing of emulsion, the mean diameter as the function of the agitation time for processing; the dotted lines explain the mean diameter for the continuous processing.

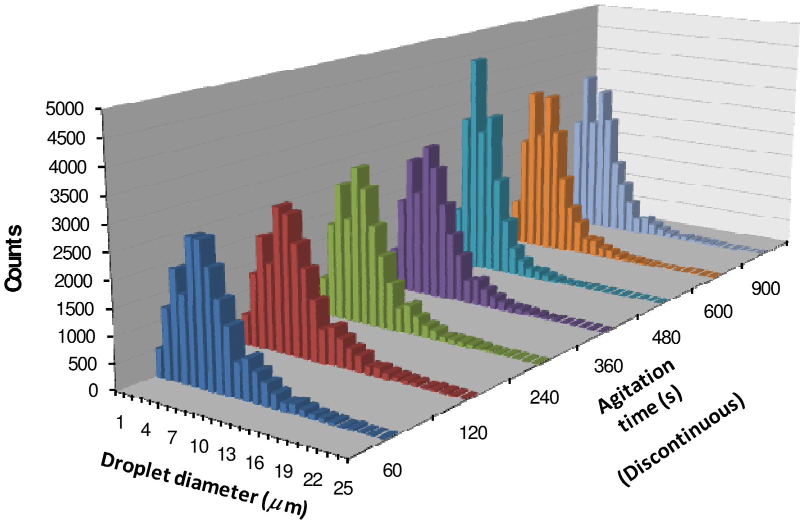


Figure 55. Comparison of Mean droplet distribution graphs of discontinuous processing

The histograms of the droplet distribution for the discontinuous processing system were represented in the Figure 54. Although a big change is not visualized in the peaks, the decline in the mean values could be observed. Figure 56 represent the mean distribution graphs for continuous processing.

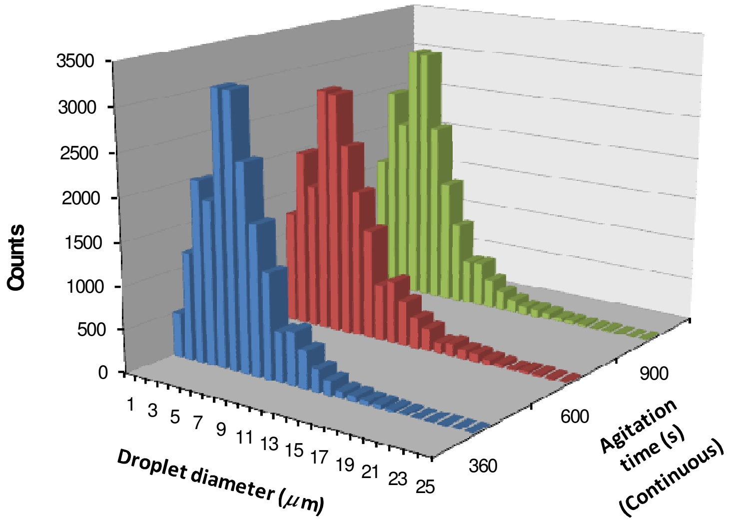


Figure 56. Comparison of mean distribution graphs on continuous processing

3.6.1.1 LD analysis

Continuous processing

The effect of continuous processing was carried out as mentioned in section 2.2.1.2. (Table 23). The same procedure was followed as carried out as mentioned in PDA analysis. Formulation (d1) was used but the speed of UT was 11500 rpm.

Table 23. Continuous processing in dilute emulsions

Agitation time (s)	(μm)	
	mean	**S.d**
10	2.133	1.58
30	1.878	1.06
120	1.272	0.90
180	0.136	0.03
360	0.142	0.05

Formulation d1, Amount of Polysorbate 80 (moles): 4.08×10^{-4}

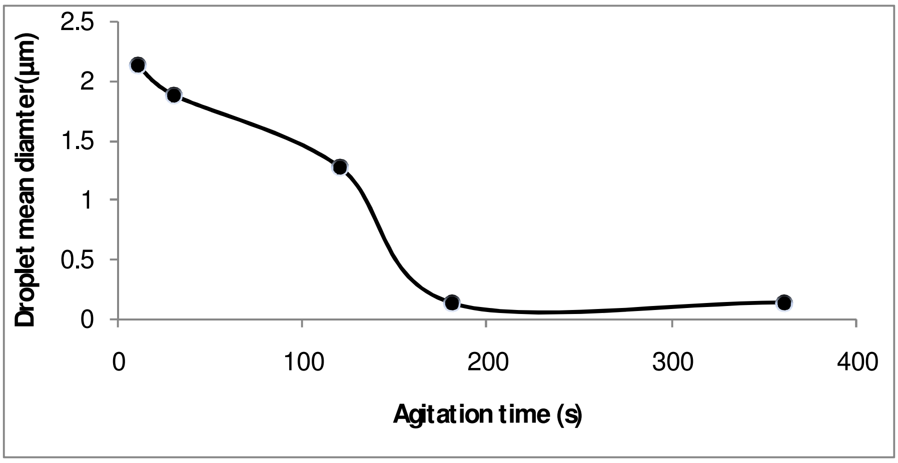


Figure 57. Agitation time versus droplet mean diameter

3.6.1.2 PDA analysis

3.6.2 Type of Surfactant

The influence of the type of the surfactant on the droplet size diameter was studied. Sorbitan monolaurate and Polysorbate 80 was compared. Sorbitan monolaurate produced larger droplets when compared to the Polysorbate 80 as the former mentioned surfactant is not suitable for the preparation of oil in water emulsions as they are mainly utilized for the water in oil emulsions. Polysorbate 80 is mainly the hydrophilic surfactant and can be used for the preparation of oil in water emulsion and they stabilize the droplets which denotes that it protects the droplets from coalescence and there by reduction in droplet size, in the case of sorbitan mono laurate it does not act as effective as Polysorbate 80 and so it may not prevent the destabilization process and the droplets are larger in size.

Table 24. Comparison of Sorbitan monolaurateand polysorbate80

Formulation	Surfactant	Amount of Surfactant		(μm)	
		ml	moles	mean	s.d
d4		0.01	$2.97\text{x}10^{-5}$	5.90	3.31
d5	Sorbitan monolaurate	0.05	$1.48\text{x}10^{-4}$	9.69	4.61
d2		0.10	$2.97\text{x}10^{-4}$	11.26	5.12
d3	Polysorbate 80	0.10	$8.16\text{x}10^{-5}$	5.94	3.72

Liquid paraffin : 0.2 ml

The above table is considered for plotting the graph of amount of surfactant alongside droplet diameter. The Table 10 was also considered for the analysis but with the formulation listed in the Table 10, (d6) the PDA could not perform the analysis, the laser could not pass through the particles as they were opaque and the emulsion appeared to be turbid. As there was an increase in the liquid paraffin volume to 0.5 ml. Laser diffraction method of analysis using Coulter counter could not perform the analysis for the formulations in Table 24.

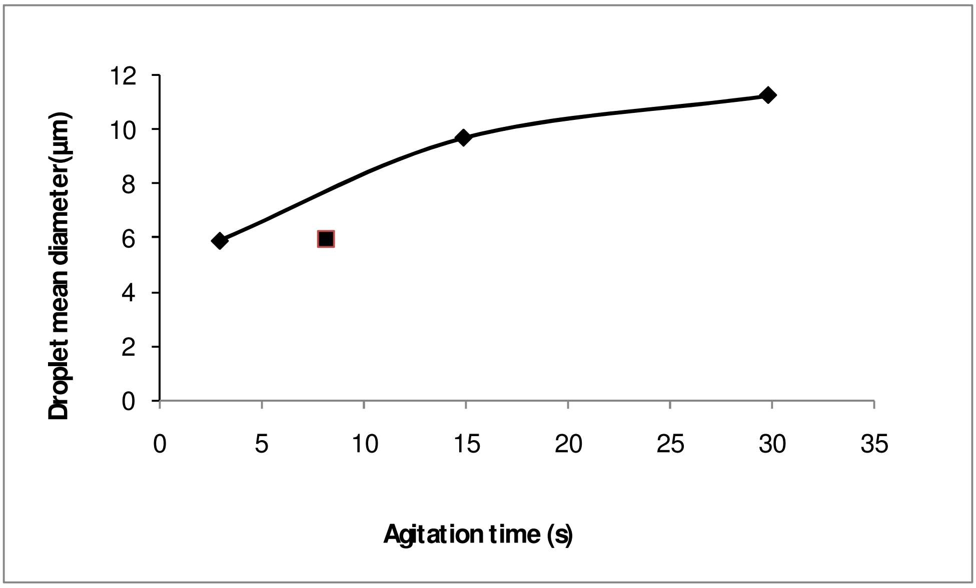


Figure 58.The effect of Sorbitan monolaurate on emulsions; [♦] Sorbitan monolaurate, [■] poly sorbate 80

The figure shows very clearly that the particle diameter increases markedly along the addition of Sorbitan monolaurate. Addition of Sorbitan monolaurate as the surfactant increases the droplet diameter compared to that of Polysorbate 80.

3.6.2.1 Heidolph agitation

The effect of the Heidolph stirrer was studied using the PDA, the formulation d1 was considered. The analysis was performed using different speed levels such as 196 rpm, 500 rpm, 1425 rpm respectively and agitation was maintained for 900 seconds through out the analysis. This analysis was done to differentiate the effect of the stirrers used. Table 25 was considered.

Table 25. Heidolph Stirrer

Formulation	Speed (rpm)	Polysorbate 80 (ml)	(μm) mean	(μm) s.d
d1h	196	0.5	5.50	3.46
d2h	500	0.5	5.48	4.12
d3h	1425	0.5	4.95	3.11

Amount of Polysorbate 80 (moles): 4.08×10^{-4}

The droplet diameter seems to decrease with an increase in the speed of the stirrer. This effect is similar to the effect of the homogenizer as discussed earlier.

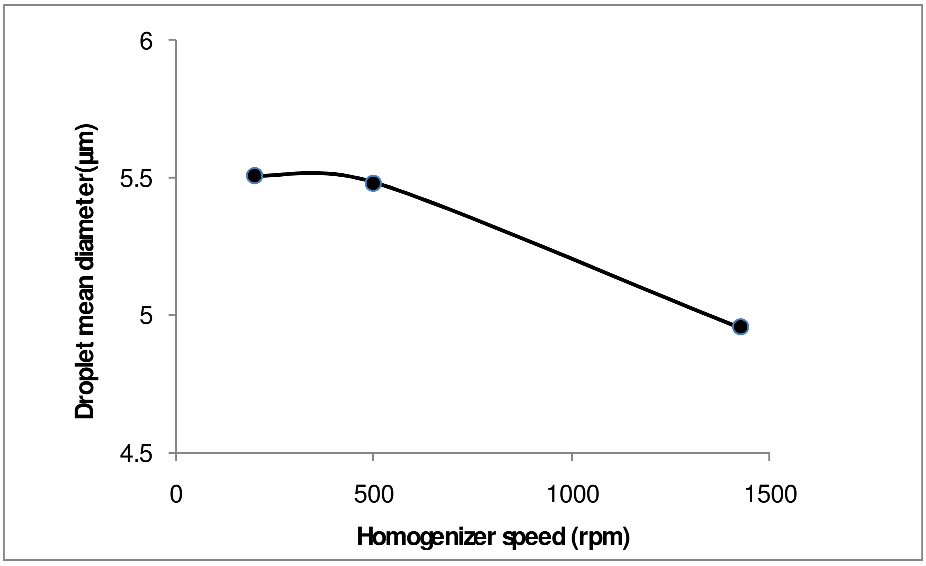


Figure 59. Heidolph agitation for 15 minutes; (47a) 1425 rpm, (47b) 500 rpm and (47c) 196 rpm

The Heidolph stirrer also confirms the earlier discussed effect of homogenizer with concentrated emulsions. The increase in agitation duration provides the reduction in droplet diameter. When the 900 seconds agitation result compared to the same study with Ultra-Turrax, these droplets were found larger than them however the size difference is only marginal where the droplets were supposed to show a bigger difference as the change in the stirrer demonstrates a change in droplet size as the maximum speed level of the Heidolph mixer was only 1425 rpm. In his study Roland et al. [24] compared different types of mixers and found that the high pressure homogenizers produced smaller droplets than other mixers used. This study is to understand the effect of the stirrer as the design and type of each stirrer influences and also crucial in determining the droplet size. Baldyga et al. [64], in his study compares his results with recent studies and concluded that the Impeller design determines the local maximum value of the time-averaged energy dissipation rate as well as the integral scale of the turbulence.

3.7 Viscosity of emulsions with varying agitation time

Measurement of viscosity is very essential mainly in characterization of the emulsion. As discussed earlier, stroke's law states that rate of phase separation between liquid (ρ_1) and liquid (ρ_2) depends on gravity (g), on the radius of the particles (r) and the viscosity of the medium (η):

$$\nu = \frac{2r^2(\rho_1,\rho_2)g}{9\eta} \quad . \qquad \textbf{\textit{Equation}}\ 17$$

The viscosity measurements were carried out using Ostwald's viscometer (refer section 2.1.5.3). The temperature was maintained constant at 22°C. The formula used was

$$\frac{\eta}{\eta_0} = \frac{m}{m_0} \cdot \frac{t}{t_0}$$

Viscosities of different emulsions were measured. It was observed that emulsion prepared with different agitation times showed a slight increase, a plateau and at 360 seconds, a fall in viscosity was observed.

Table 26. Viscosity on increasing agitation time

Formulation	Agitation time (s)	Viscosity (mPas)	Density
1	10	4.72	1.013
2	20	6.40	1.017
3	30	6.40	1.017
4	60	8.97	1.015
5	120	8.92	1.015
6	180	8.92	1.019
7	300	3.46	1.017
8	360	2.94	1.017

pH = 6.22; Temperature = 22ºC

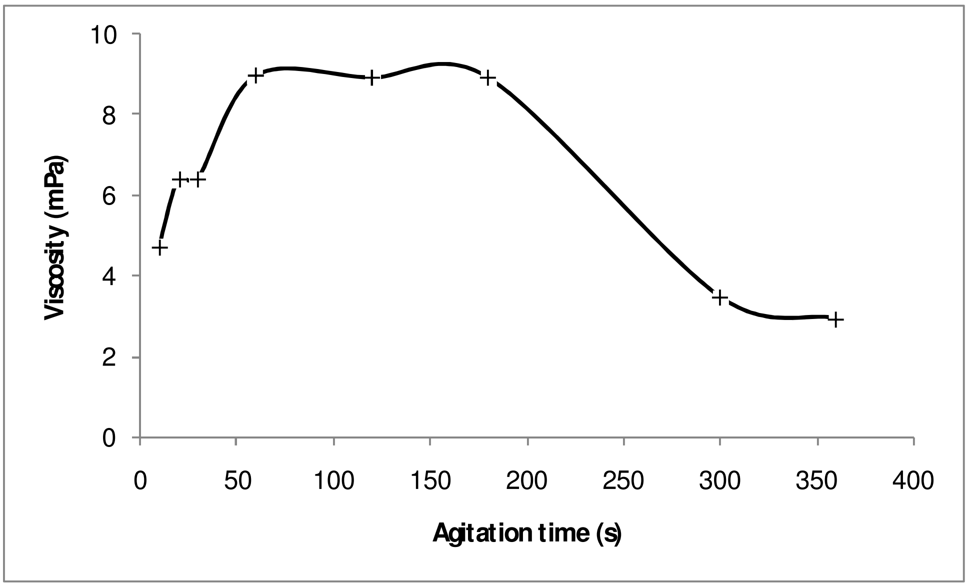


Figure 60. Viscosity with increasing agitation time

The increase in agitation time causes formation of homogeneous droplets thus increasing the viscosity of the emulsion.

The above graphical representation shows an elevation in viscosity as the function of agitation time, the droplet diameter increases along the agitation up to 60 seconds. From that stand point it slides down consistently probably due to pseudoplastic behaviour.

3.8 Viscosity on emulsions with different surfactant volumes

Addition of surfactant increases the viscosity to a great deal. The surfactant improves the surface viscosity and reduces the interfacial tension. It reduces the movements of the interface and slows down coalescence and makes the emulsion more stable.

Table 27. Viscosity with different surfactant voumes

Formulation	Polysorbate 80(ml)	pH	Viscosity (mPas)	Density
19	2	6.22	2.948	1.017
20	5	6.35	3.200	1.011
21	10	6.32	11.310	1.017
22	20	6.31	60.210	1.014

Liquid paraffin: 50 ml ;Water: 50 ml; Agitation time: 360 s

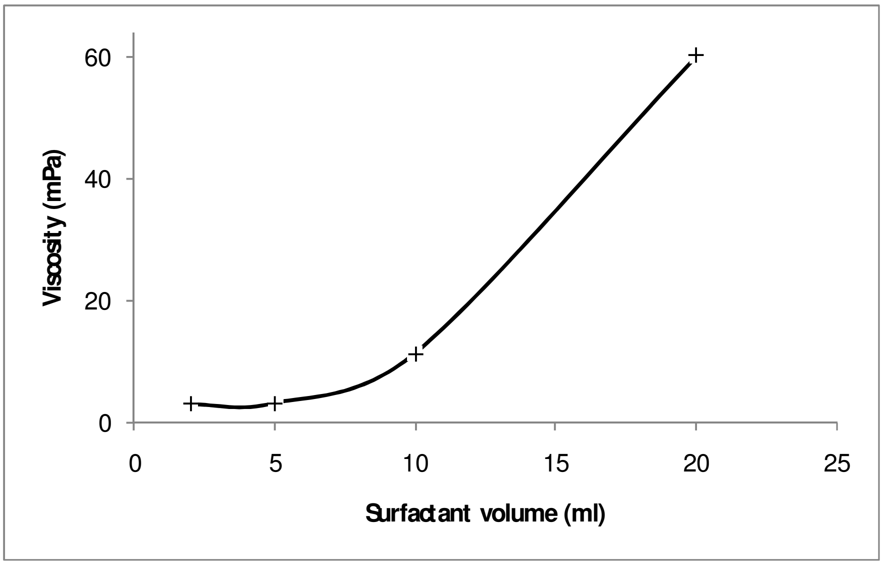


Figure 61. Viscosity with increasing surfactant volume

Figure 61 shows a fair progress in the emulsification as the increase in viscosity is mark of stability. The Surfactant concentration increases the narrow distribution of droplets and thus improves the viscosity. This results stays in pace with that of Behrend et al. [19], where he stated that stabilizers lead to improved droplet disruption at constant energy density.

This increase in viscosity with increase in surfactant volume stay comparable with the results published in the review by Pal [65], where it was stated that presence of a thick adsorbed layer of surfactant at the particle surface can result in an increase in viscosity of the system.

3.9 Viscosity of emulsions with different oil volumes

Table 28. Viscosity with different Oil volumes

Formulation	LIQUID PARAFFIN (ml)	pH	Viscosity (mPas)	Density
23	40	6.32	26.36	1.022
24	50	6.31	63.06	1.014
25	60	6.31	62.76	1.016
26	70	6.29	81.70	1.015

Liquid paraffin: 50 ml; Water: 50 ml; Polysorbate 80: 20 ml
Agitation time: 360 sec; speed: 11500 rpm

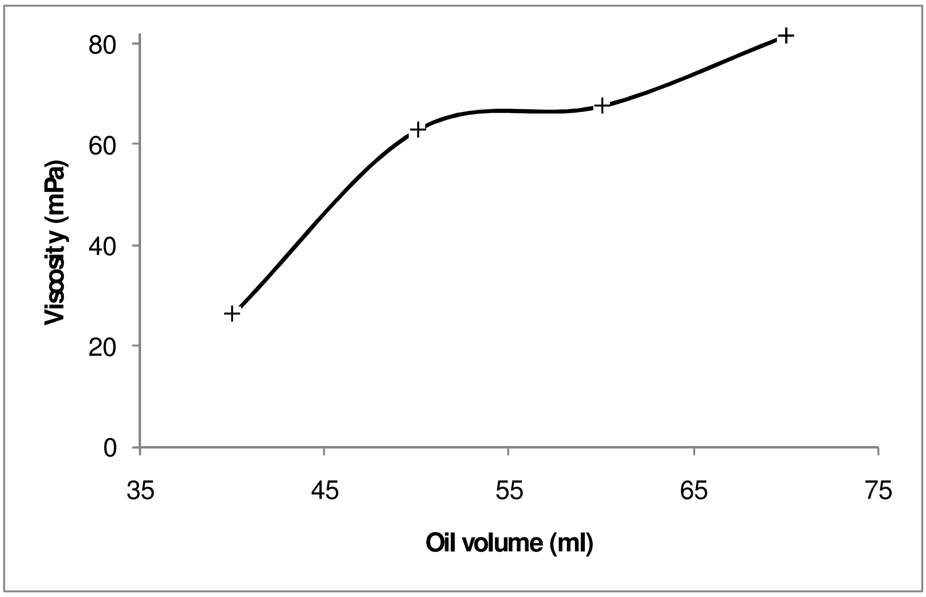


Figure 62. Viscosity change on increase in oil phase volume

The above graphical representation signifies a prominent increase in viscosity with an increase in oil phase volume. The graph denotes a relevant point as the oil increases the viscosity of the emulsion to a great extent. However in the Figure 62 we could see that the rapid increase in viscosity declines in small degree when the formulation 25 was considered, still there was no hold back it continues to increase up to 80 mPas. Thus proves increase in oil volume increases the viscosity. Similarly in accordance with the obtained result Dlużewska et al. [66], concluded that increase of oil phase concentration from 20% to 40% independent of the kind of the emulsifier used, caused an increase in apparent viscosity of the emulsion.

3.10 Viscosity of emulsions on ageing

The viscosity was analyzed for the formulation (1) by ageing it to study the effect of ageing on viscosity of the emulsion. On ageing the viscosity decreases drastically. Ageing causes increase in droplet diameter and the surfactant molecules are not adsorbed to the interface causing reduction in viscosity. Figure 63 shows a consistent fall in viscosity as the emulsion starts to age.

The results obtained were different when compared with the results published in the review of Pal [*65*], here he has discussed that at low shear stresses the viscosity of o/w emulsions decrease initially with aging but the decrease is only marginal

With further increase in age, the viscosity tends to increase to the original level. But here in this study the results show the persistent decrease in viscosity.

Table 29. Viscosity along ageing

Ageing after (min)	Viscosity (mPas)	Density
5	2.990	1.020
15	3.127	1.019
30	3.011	1.017
60	2.620	1.006
120	2.600	0.997
180	1.960	0.999
220	1.910	0.999

Liquid paraffin: 50 ml; Water: 50 ml; Polysorbate: 2 ml
Formulation: 1; Agitation time: 360 s; pH: 6.22

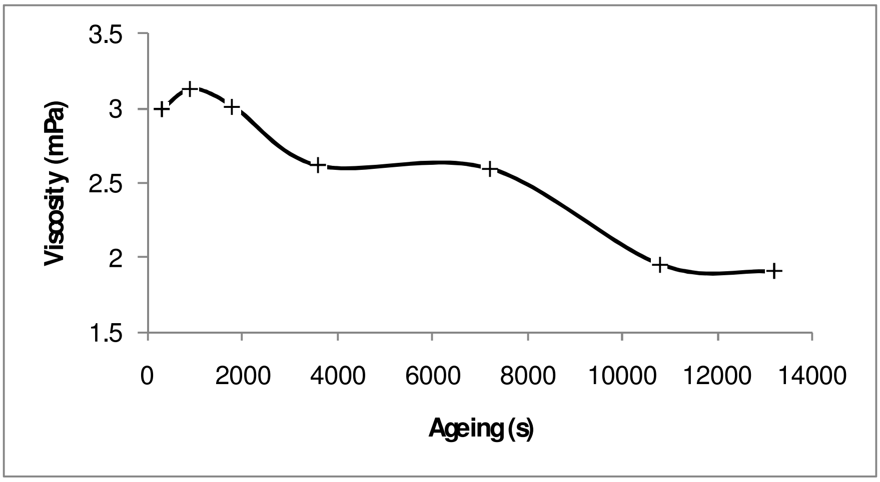


Figure 63. Viscosity on ageing

3.11 Viscosity of dilute emulsions

The viscosity of the dilute emulsions is found to be very less. On increasing the water (continuous phase volume), the viscosity decreases. The result is in good agreement with the results of Shigemoto et al [67]. The relative viscosity decreases with increase in water content irrespective of the surfactant used.

Table 30. Viscosity of the dilute emulsion

Liquid paraffin (ml)	Polysorbate 80 (ml)	Surf-oil ratio	Water (ml)	pH	Viscosity (mPas)	Density
0.2	0.5	2.5	50	5.55	0.907	1.077

Considering the methods used for the droplet size measurement, PDA fits better as the real time analyzer as it is a dynamic measuring technique whereas optical microscopy seems to be a static measuring technique and also tedious one. Photon correlation spectroscopy possesses limitations like higher dilution and the size range boundaries. Laser diffraction is also an off-line technique where the sample has to be made and taken to the equipment and needs sampling procedure.

From this study, it agrees with studies stating that PCS measurements appeared to be less reliable for the larger particles of 2 µm. Jaeger et al. [68], analyzed the possibilities and limitations of Photon correlation spectroscopy, he studied polystyrene lattices and concluded that for particles less than 0.5 microns reliable data was obtained and with particles ranging between 0.5 to 1 micron the reliable results were for only narrow range of concentration. For particles about 2 microns the PCS method was less reliable and displayed variation of results. This study goes in good agreement with the results obtained from PCS showing variation of sizes compared to other methods. When we consider the Laser diffraction method the Fraunhoffer diffraction theory applies to particles whose diameter is considerably larger than wavelength of illumination. Therefore the accuracy of the particle size distribution by Laser diffraction is suspect when the dispersion contains a large fraction of particles lesser than 10 microns. For smaller diameters Mie theory is more accurate and useful.

Microscopy is an excellent technique that allows direct examination of the particles however, it is not suitable as a quality or production control technique beyond the level of simple judgment. Also, as relatively few particles are examined, there is a real danger of unrepresentative sampling and if weight distribution is measured results are magnified. Missing one 10m m particle has the same effect as missing one thousand 1m m particles. Roland et al. [24], suggested that to efficiently assess the stability of an emulsion and to characterize the formulation, Turbiscan analysis, Stability under centrifugation etc can be carried out and further added that Microscopy analysis may be superfluous as it could not be used for submicron emulsion droplets.

Phase Doppler Anemometry (PDA) technique do have limitations of the size range, the lower limit is around 0.5 microns but it could measure even up to 100 micrometer effectively as the upper limit is high. Nevertheless PDA gives us a scope to monitor online the droplets, there exists an opportunity to get a continuous view of the process as specified earlier under the section 1.3. PDA could be used to perform dynamic evaluation on droplet measurement. Occurrence of any unfavorable factor could be altered by changing the processing parameters which are critical for obtaining the desired product.

The results obtained with PDA seem to be consistent and the equipment responds to the different changes put in as different effects were studied in a consistent way. Unlike Photo correlation spectroscopy which delivered results in a particular size range however this was the problem due the experiments done in the limiting range of the equipment. Microscopy study showed higher mean values when compared to other techniques. The reason may be due to fairly less dilution of the emulsion with this method because the dilution of the sample may contribute to reduction in droplet diameter as dilution may result in changes in the droplet size distribution associated with break up, coagulation or coalescence [51]. It can be put that PDA offers feasibility for the formulator to “Act Right at the first time” as one can adjust or alter the homogenization speed or agitation time duration during the emulsification by the supervising the signals reflecting the droplet size.

Chapter 4 CONCLUSIONS AND FUTURE WORK

4.1 Conclusions

Following the study conducted it is relevant that all the three equipments phase Doppler anemometry, photon correlation spectroscopy and microscopy provides a uniformity in a pattern developed during each parameter study like effect of agitation, homogenization efficiency, surfactant effect and oil effect, ageing etc. Although the values from PCS may not be relevant, the pattern obtained with the study was on par with the other two methods. The processing experiments were conducted for dilute emulsions by measuring the emulsion droplets discontinuously in order to study the kinetics that may change due to the sudden arrest of agitation and to the contrary the continuous agitation performed without any stop but the comparison does not reveal to be very significant although it showed a trend that discontinuous processing declines, the continuous processing does not really show a big decline as expected. The Sorbitan monolaurate when compared to Polysorbate 80 seemed to produce larger droplets due to the fact it is not hydrophilic and so it may not be available enough at the interface to avoid coalescence. Polysorbate 80 could not be employed in all three tests like sorbitan monolaurate because polysorbate 80 at the same compositions poses problems for PDA for measuring due to opacity. Effect of the mixer type studied using Heidolph showed a decline along the increase in agitation rate but the effect of a different mixer to study the difference between Ultra Turrax and Heidolph could not be achieved as the droplet size change between them were not significant. The emulsions were all prepared at room temperature. The tests of viscosity and pH of the emulsions can be the criteria for the improvement of quality control.

Concerning the methods employed for the studies as discussed enough earlier PDA deserves a better scope to be developed as the still better online monitoring tool which needs to be improvised as the field equipment. Various other techniques discussed techniques like FBRM, Turbiscan® seems to be very promising. On quality control basis to choose a particle sizing method, the method has to be judged up on technique's ability to detect a significant change compared to previous testing. To ascertain the robustness of the method the validation of the analysis is very important. The implementation of validation study becomes absolutely important in pharmaceutical manufacture. Validation offers the result consistency which is the crucial to ensure efficient production and quality control. The development of validated method should be carried out with appropriate software and is regularly tested to confirm its performance.

4.2 Future work

The future work may involve optimization of an experimental procedure for obtaining an emulsion with specific desired type. By considering chemometrics an experimental design can be developed. All the stability tests for the discussed product including Zeta potential has to be done. Drug modeling can be carried out by incorporating an active principle. The concentrated emulsion can be diluted and phase Doppler anemometry can be involved in the online sizing of the emulsion droplets. Direct preparation of the emulsion by setting the homogenizer apparatus in line on the top of the measurement compartment can be facilitated.

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