Review Article

Nanoemulsion Formulation Techniques, Characterization and their Pharmaceutical Applications: A Comprehensive Technical Review

ABSTRACT

Background: This review discusses the development, manufacturing, fabrication, and manipulation of nanoemulsions, an advanced drug delivery method that addresses the limitations of conventional systems. Nanoemulsions are biphasic dispersions of immiscible liquids, either water in oil or oil in water, stabilized by an amphiphilic surfactant. They offer various drug delivery functionalities but face challenges in stability, structure control, and characterization. Nanoemulsions, with droplet sizes of 100 nm, are kinetically stable liquid-in-liquid dispersions with high surface area, robust stability, optical transparency, and tunable rheology. These submicron-sized emulsions are being studied for drug delivery and targeting, offering potential in cosmetics, diagnostics, drug therapies, and biotechnologies. They are used in cancer treatment, drug targeting, mucosal vaccines, transdermal drug delivery, lipophilic drugs, and self-nanoemulsifying drug delivery systems.**Objective:** This review explores various techniques for developing and characterizing nanoemulsions, their formation and stability theories, and their current and future applications due to their unique structures and chemistries. This review discusses the importance of optimal formulation for nano-droplet systems, focusing on droplet size, solubilization, colloidal stability, optical and rheological properties. **Conclusion:** This research focuses on the study of various techniques of preparing nanoemulsions i.e., high energy methods and low energy methods. This study explores the best methods for formulating nanoemulsions, their characterization, release kinetics, and application in various fields.

Keywords: Nanoemulsion, High Energy Homogenization, Low Energy Homogenization, Drug Delivery System, Dispersion System

Comment [U1]: Review

Introduction

Nanoemulsion drug delivery systems are a promising tool for delivering and improving the bioavailability of hydrophobic drugs and bioactive food components in the blood. The majority of drugs are hydrophobic (lipophilic) in nature, thus leads to low solubility and bioavailability problems ;the bioactive food components also show low bioavailabilities in conventional doses [1,9,49].In the food industry, nanoemulsions are being explored to encapsulate, stabilize, and deliver lipophilic constituents like flavors, omega-3 fatty acids, vitamins, preservatives, nutraceuticals. They have a number of potential advantages over conventional emulsions like incorporation into optically transparent products, may enhance the texture, stability, and bioavailability of products[2-5].A widely used high energy method to reduce the droplet size of nanoemulsions is ultrasonication. In this method, mechanical vibrations from ultrasound waves (> 20 kHz) create sinusoidal pressure variation in the emulsion system[6,7,50]. The objective of this review are to explore various techniques for developing and characterizing nanoemulsions, their formation and stability theories, and their current and future applications due to their unique structures and chemistries.

FORMULATION TECHNIQUES OF NANOEMULSION

High energy methods

1. High-pressure homogenization

Nanoemulsions are often produced in high-pressure homogenizers.

Construction

- A pump used in high-pressure homogenizers increases the dispersion's pressure by 500–5000 psi.
- The homogenizing valve's opening through which fluids are forced.[1]

Process

A coarse emulsion is formed using a high shear mixer and introduced into a high-pressure homogenizer, resulting in a fine emulsion. Forces like turbulence, shear, cavitation, shock, shear stress, pressure gradient, and expansion shear cause droplet breakage. [2]The homogenizer employs various nozzle types to enhance droplet fracturing, resultin a nanoemulsion with a lipophilic core separated by a monomolecular phospholipid layer.[3]The final product undergoes

hydraulic shear and turbulence, forming a small particle emulsion, effectively reducing the size of coarse emulsions by mixing oil and water separately.

Operational parameters

The droplet size decreases with increasing homogenization pressure, emulsifier adsorption rate and interfacial tension, and increases due to the decrease of these factors.

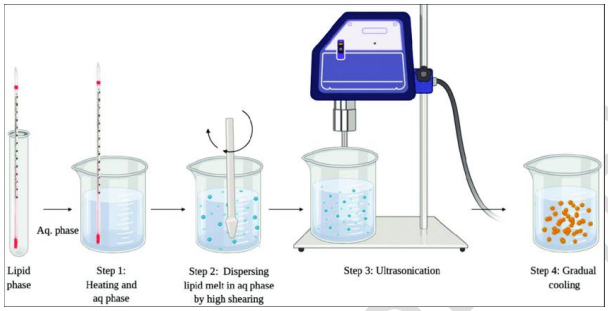

Figure 1.Formulation of nanoemulsion using cold high-pressure homogenization technique[4]

Merits

- The homogenizer produces smaller particle sizes by delivering the power in the shortest possible time with the most homogeneous flow (up to 1 nm).
- High efficiency

Demerits

- Heavy reliance on energy
- An increase in emulsion temperature during processing.

Examples of high-pressure homogenization

I. Preparation of D-limonene organogel and organogel-based nanoemulsion by high-pressure homogenization

The study involved mixing an organogelator with D-limonene, MCT oil, and stearic acid or monosterine to create organogels. A high-pressure homogenization method was used to create a nanoemulsion based on D-limonene organogel. The emulsion was then homogenized under high pressure. Various batches were prepared to evaluate their effects on formulation formation and stability.[5]

II. Isoflurane-loaded nanoemulsion prepared by high-pressure homogenization

The aqueous and oily stages of the process required separate preparation steps. First, 15 mL of medium-chain triglycerides were dissolved in Lipoid S75® (1% w/v) at 30°C while being

magnetically agitated. Before the emulsion formed, ISO (15 mL) was added after the liquid had been cooled to 18°C. 64.5 mL of water, 2.5% sorbitol by volume, and 2% polysorbate 80 by weight were mixed for the aqueous phase at 30°C with magnetic stirring and then cooled to 18°C for emulsification.A high-shear mixer (Ultraturrax®, IkaLabortechnik) was used to emulsify the oily and aqueous phases for one minute at 16,000 rpm, producing a coarse emulsion. A high-pressure homogenizer was used to further treat this emulsion at 10 °C using the output cooler[6]

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2. Ultrasonication

Ultrasonication is a high-energy method used to decrease droplet size in emulsions by causing a sinusoidal pressure shift due to mechanical vibrations above 2kHz.[7]

Construction

A piezoelectric probe is used to produce a strong disruptive force by the help of its tip.

Process

The process involves mixing a homogenous oil phase into an aqueous phase, creating a coarse emulsion, and then subjecting it to ultrasonication to create nanoemulsions. Bubbles are produced through cavitation, causing droplets to condense.

Merits

Less energy expenditure.

Demerits

Contamination caused by probe.

Operational parameters

Increased sonication time and input power decrease droplet size. Probe placement, depth, and contact with solid surfaces affect pressure distribution and wave reflection.[8]

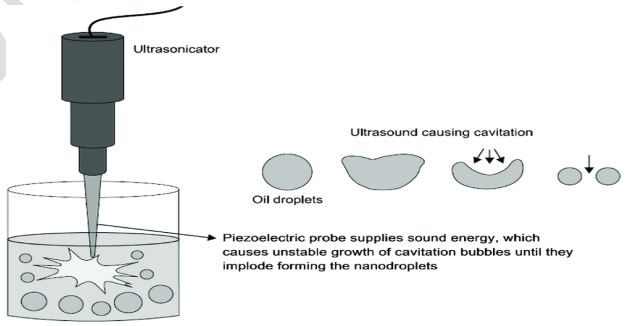

Figure 2. Ultrasonication technique [9]

Example of ultrasonication method

1. Preparation of a novel curcuminnanoemulsion by ultrasonication

CurcuminNanoemulsion (Cur-NE) was created using high-energy ultrasonication, dissolved in oil, combined with surfactants, and titrated with Milli-Q water to create a coarse emulsion. Using a 20kHz ultrasonic processor, the final nanoemulsion was produced with the following settings: 40.0% ultrasonication intensity, 10.0 minutes of ultrasonication time, and 50°C temperature. The process was facilitated by cavitation, a high-intensity ultrasonication phenomenon, resulting in Cur-NE with improved Brownian motion and smaller globule sizes for extended storage.[10]

3. Microfluidization

the droplet size of the previously formed coarse emulsion is reduced by using microfluidizers[11] the working mechanism for size reduction includes hydraulic shear, impact, attrition, impingement, intense turbulence and cavitation.[8]

Construction

A microfluidizer consists of

- An Inlet feed
- A pressure intensifier pump
- An interaction chambers
- Cooling coil
- Outlet

Process

The pre-emulsion feed is divided into two channels using a stainless-steel block, which are positioned inside the device. The channel of the block gets smaller until it is about 75 µm wide. the two feeds are made to collide directly. As a result, a very high shear is generated, resulting in the production of nanoemulsion.[11]

Operational parameters

The energy input can be increased by adjusting the operating pressure or emulsification time, which can be achieved by repeating the procedure multiple times**.**[11]

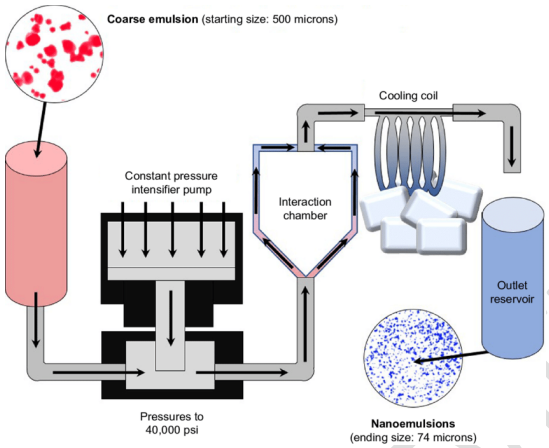

Figure 3.Microfluidization Technique [12]

Merits

No contamination of feed material [8]

Continuous mass production of large quantities of goods and preparations is possible.[13]

Demerits

- As the emulsification begins quickly, the biopolymers used in this method are unable to stabilize recently disrupted droplets in that short amount of time
- High energy density that is produced during the procedure.

In "over-processing," the effects of droplet disruption are amplified by recoalescence, leading to an increase in EDS (Energy Density Spread), when the energy density is raised above the optimum level.[11]

Example of microfluidization method

Production of sub-micron emulsions by ultrasound and microfluidization techniques

Previously prepared coarse emulsions (at room temperature) were passed through an air-driven microfluidizer (Model M-110 L, Microfluidics, USA). The system was fed pre-emulsion via a 200 mL glass reservoir. The device divides the pre-emulsion feed into two opposing channels in a stainless-steel block (a ceramic interaction chamber); these channels narrow to about 75 ml in width, and the two jets of pre-emulsion collide head-on at high pressure, resulting in extreme shear. When the air pressure at the regulator is 530 kPa, the typical pressure of the liquid jets flowing through the channels is about 120 MPa due to mechanical amplification of 232. The

emulsions' volume flow rate was 4 × 10 ^ -6 m3/s 60 MPa for one cycle. The experiments were duplicated.[11]

Low energy methods

Low energy methods use internal physical properties of the system such as temperature or composition to produce nanoemulsions[14]

4. Phase inversion by temperature

The process involves heating surfactant, oil, and water, stirring until cool, to produce nanoemulsions with small droplets and narrow particle sizes, ensuring high reliability and consistency.[14]

Working principle and mechanism

The PIT method uses temperature fluctuations to alter the hydration characteristics of nonionic surfactant head groups. At low temperatures, the head-group is highly hydrated, while at high temperatures, it becomes dehydrated. This affects the surfactant's water solubility and ideal surfactant monolayer curvature.[14]

Process

Phase inversion temperature determination (PIT)

The hydrophilic-lipophilic balancing temperature (HLB) was calculated using electrical conductivity method. Mixtures of decane, water, and surfactant were agitated, and the HLB temperature, or phase inversion temperature, was determined by heating.[15]

Emulsification by phase inversion by temperature

There are two steps involved. Initially, a temperature up to 15 C higher than the PIT corresponding to the given surfactant concentration was achieved by simultaneously and separately heating the water phase and the oil phase containing the surfactant. When the oil phase reached that temperature, water was added, and the mixture was removed from the heat source and allowed to cool naturally before being transported to the PIT.

The oil phase was solubilized into a bicontinuousmicroemulsion at the HLB temperature, then chilled to 25 degrees Celsius and continuously stirred during chilling.[15]

Process variables

Surfactant concentrations effect the PIT, droplet size, particle morphology and storage stability

oil phase composition affects the turbidity which have direct impact on the PIT.[14]

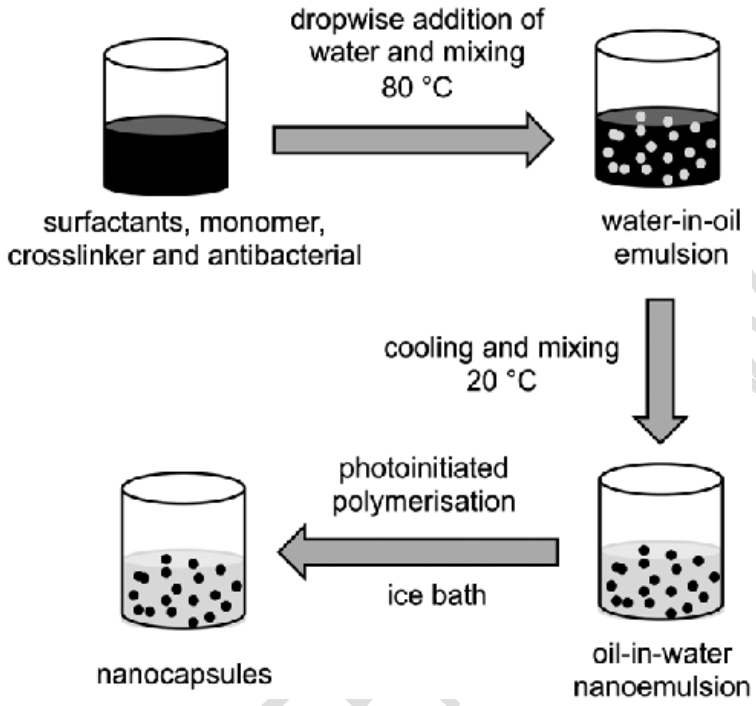

Figure 4. Mechanism of nanoemulsion formation by PIT method [14]

Merits

- It is simple to perform and implement.
- There is no need for sophisticated equipment.

Demerits

- Higher surfactant concentrations cause instability problems.

Example

1. Preparation of Cinnamon nanoemulsion by phase inversion by temperature method

The ratio of cinnamon oil to MCT was changed from 0:10 to 1:9 to 2:8, 3:7 to 5:5, 6:4, 7:3, 8:2, and 10:0 while keeping the overall amounts of the oil phase (10 wt%), surfactant (10 wt%), and water phase (80 wt%) constant. Tween® 80 and deionized water were added after three minutes of blending cinnamon oil and MCT. The study involved combining components for 30 minutes, heating each solution to 15°C, determining PIT for minimal turbidity, and cooling twice.Initially, cooling to the PIT temperature may result in the formation of a stable microemulsion. The system was then rapidly cooled by adding 250 g of cold, deionized water (4°C) and stirring

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continuously for three minutes. The study aimed to determine the mean droplet diameter, stability, and particle size distribution of each sample by rapidly cooling it to the PIT temperature.[14]

5. Solvent displacement method

This method involves combining the organic phase—which contains the oil dispersed in a solvent such as ethanol or acetone—with the aqueous phase, which contains the surfactants.[16]

Working principle and mechanism

Emulsification occurs spontaneously due to diffusion of organic solvent, which may be removed later by vacuum evaporation.[16]

Process

The solvent displacement method precipitates polymers from an organic solution, dissolves them in a semipolar solvent, and then adds it to an aqueous solution containing stabilizer, forming nanoparticles instantly**.** [17]

Process variables

The choice of emulsifying device is influenced by factors like volume, viscosity, surfactant type, temperature, and droplet size, with optimization of formulation parameters achieving desired nanoemulsions**.**[16]

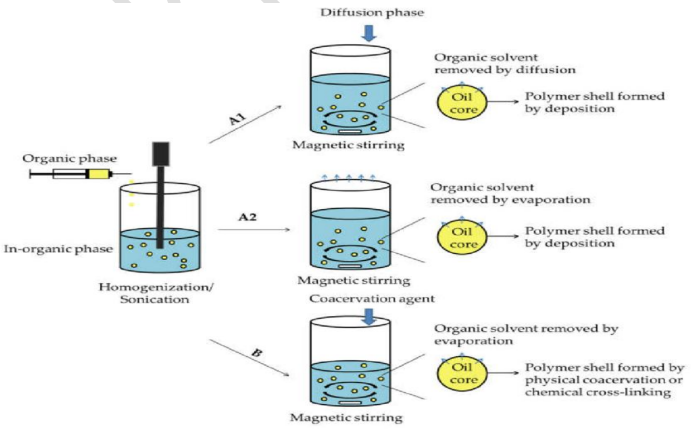

Figure 5. Representation of Solvent Displacement Method [18]

Merits

- Requires no heating
- No requirement of an organic solvent and LC or ME as a phase

Demerits

The need of a large ratio of solvent and oil for the production of small sized droplets of the disperse phase.[16]

Example

I. Preparation of maltodextrin-stabilized a-tocopherolnanoemulsions using the solvent-displacement technique

The study created a-tocopherolnanoemulsions using solvent-displacement and different ratios of Polysorbate 20 and maltodextrin, using pure deionized water and diluted Polysorbate 20 with purified water. A-tocopherol was dissolved in acetone at a concentration of 1% w/v to prepare the organic phase. For all samples, the organic-to-aqueous phase volume ratio was 1:1. The organic phase and Polysorbate 20 solution were slowly added to the maltodextrin solution under conventional homogenization at 15,000 r/min for 10 minutes after the solutions had been homogenized using magnetic stirring. Finally, acetone was extracted from the system using a rotary evaporator set to 30 degrees Celsius for 20 minutes.[19]

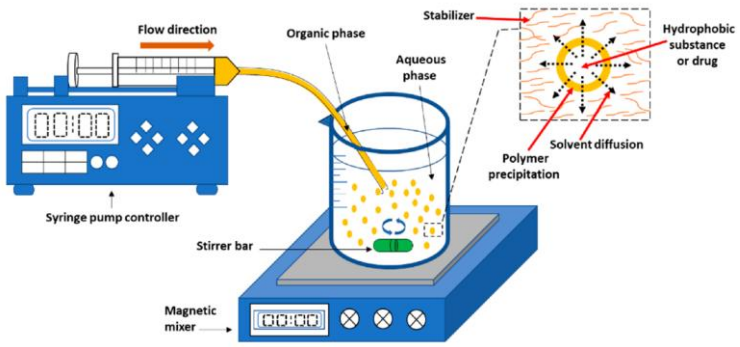

Figure 6.The nanoprecipitation process is illustrated in a diagram[20]

6. Self-Emulsifying Nanoemulsion (SNEDDS)

SNEDDS are isotrophic mixtures of oil, surfactant, co-surfactant, and drug that form aqueous oil-in-water emulsion with little stirring.[21]

Process and mechanism

SEFs are mixtures of oil, surfactant, co-surfactant, and co-solvents that form a transparent, isotropic solution that emulsifies under gentle agitation, similar to the gastrointestinal tract. (GIT).

Variables

There are many factors that affect SNEDDS

High-dose drugs are not suitable for SNEDDS due to their limited water solubility and difficulty in delivering lipids. SNEDDS' solubility in the oily phase is crucial, and larger surfactant or co-surfactant roles can lead to precipitation.

Merits

- Increased consistency in drug absorption.
- Drug(s) are selectively targeted toward a specific absorption window in the GI tract.
- Drug(s) are shielded from the gut environment.
- Delivery profile management.
- Variability has been reduced, including food effects.
- Increased oral bioavailability allows for dose reduction and high drug loading efficiency.
- Low production costs
- increased stability

Demerits

- There are no good predictive in vitro models for evaluating formulations.
- Traditional dissolution methods are ineffective because formulations are not dependent on digestion prior to drug release.
- Further development and validation of the in vitro model is required. Various lipid-based prototype formulations must be developed and tested in vivo.
- GIT may be irritated by chemical instabilities of drugs and high surfactant concentrations in formulations (approximately 30-60%).
- Volatile cosolvents may migrate into the shells of soft or hard gelatin capsules, causing lipophilic drugs to precipitate.
- The dilution effect of the hydrophilic solvent may increase the precipitation tendency of the drug. Validation of multi-component formulations becomes more difficult.

7. A Review on Self Emulsifying Nanoemulsion

BCS Class IV Drugs

Class IV drugs pose challenges in oral formulations due to low solubility and permeability. Self-emulsifying drug delivery systems (SEDDS) can increase solubility and permeability by increasing drug concentration, inhibiting enzymatic breakdown, and promoting intestinal lymphatic transport[22]

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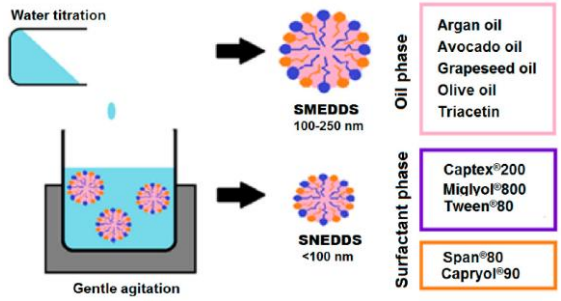

Figure 7.Self-Emulsifying Method[23]

Example of self-emulsifyingnanoemulsions

i. Self-Nanoemulsifying Drug Delivery System of chlorpromazine

Chlorpromazine (log P = 5.6, aqueous solubility 2.55 mg/L) is one of the most important and effective anti-psychotic and anti-emetic drugs in the phenothiazine derivative class. Chlorpromazine, classified as class IV in the BCS, is a dopamine antagonist with low oral bioavailability due to variable oral bioavailability and significant first-pass metabolism. To improve bioavailability, it should be packaged with a delivery system. [24]

ii. Preparation of Chlorpromazine Nanoemulsion by Self-Emulsification Method

The solubility of chlorpromazine in oils, surfactants, and ethanol was studied to create SNEDDS. Formulations were created by adding glycerides and surfactants to a 20 mg dose, heating, and adding ethanol. After 48 hours, formulations were checked for phase separation, turbidity, and particle size characterization. [24]

CHARACTERIZATION OF NANOEMULSIONS

1. Droplet size

Transmission electron microscopy

Introduction

To investigate form and size of nanoparticles transmission electron microscopy is used. For this we use 300 mesh copper formvar / carbon TEM grid with glow discharge. Samples are prepared by incorporating dilute solutions and then drying at room temperature. For TEM only most stable emulsions can be used.[25]

Method

The specimens were positioned on a polycarbonate base, and any surplus water was allowed to evaporate naturally at room temperature (25 ± 8°C). Subsequently, they undergo drying in a critical point dryer utilizing carbon dioxide, followed by sputter coating with gold using a metallizer. Finally, the samples were scrutinized using a scanning electron microscope with an operational accelerating voltage of 20 kV.[26]

2. Atomic force microscopy

Introduction

The AFM technique, due to its ease of use and similarity to real nanoemulsion systems, has become a preferred method for characterizing the interfacial properties of nanoemulsions.This techniqueisto examine physical attributes of nano emulsified coatings as average roughness, root mean square roughness, surface morphology and droplet size of nanoemulsions.[27]

Method

It is essential to dilute most nanoemulsions with distilled water between 100 and 1000 times in order to prevent droplet aggregation and coalescence. Subsequently, the diluted solution is spread over the previously split mica substrate. Before dehydrating, the accumulated droplets are sometimes cleaned with distilled water and kept at room temperature for a whole night in a dust-free environment. Alternatively, the drying process can be accelerated up by employing a furnace or heater. Adsorption occurs when charges on a sample and mica surface are attracted due to droplet binding on the substrate surface. The size and shape of the droplets were then determined using the images taken shots [27]and then observed by Atomic force microscopy having resolution upto 0.1 nm.[25]

3. Interfacial tension

Introduction

Interfacial tension is used to study formulation and characteristics of nanoemulsions. When surfactant phase is in equilibrium with oil and aqueous phases, ultralow levels of interfacial tension are indicated with phase behavior. Extremely low levels of interfacial tension can be determined by spinning drop equipment.[28]

Method

Nanoemulsions droplet size is measured by photon correlation spectroscopy. It is done by using a volumetric flask in which 0.1ml formulation and 50ml water is added and mixed by inverting flask gently. Measurements is taken by setting zeta sizer and light scattering monitor at 25 C at specific angle (90 or 180). [29]

4. Zeta potential

Introduction

Zeta potential is a measure of particle charge which is important characteristic to determine stability of nanoemulsions.High zeta potential indicate stability which means solution show resistance to aggregation. Low potential indicates attraction exceed repulsion and dispersion flocculates. This measure indicates forces between particles at nanoemulsions surface which helps in stabilization of nanoemulsions. For electrostatically stable emulsions zeta potential must be 30 mV. For nano scaled particles zeta potential influenced by manufacturer such as particle source, electrolyte concentration, pH, hydration, particle morphology. [25]

Method

Zeta potential was determined by using the Electrophoretic mobility of particles in an electric field using Zeta sizer Nano ZS Apparatus. Zeta potential of the formulation was measured using Beckman Coulter Delsa Nano C Particle analyzer, USA. Through the determination of the electrostatic magnitude and the repulsion or attraction charge between particles, the potential value of zeta gives an indication of the stability of the nanoemulsion. To maintain stability, an emulsion has to attain a minimum of 30 mV (positive or negative) of zeta-potential value. [30]

5. Polydispersity index and Particle size

A spectrophotometer is employed for this purpose to determine consistency of droplet size of nanoemulsions. It represents standard deviation to mean droplet size ratio. Decreased consistency will increase polydispersity.[28]

Method

In order to circumvent the effects of multiple scattering, the measurement of PDI and z-averages (mean particle size) was performed using ultrapure water (1:10) and Zeta sizer Nano-ZS (Malvern Instruments Ltd.[31] Prior to measurement, the samples were diluted, and 25°C and 1.33 were selected as the temperature and water's respective refractive indices. The averages and standard deviations of the three measures were noted. The PDI is used to describe the size droplets distribution width. According to a value between 0.1 and 0.25 indicates a restricted size distribution, whereas a value greater than 0.5 indicates a broad range of particle sizes.[30, 32]

6. Refractive index

Introduction

Refractive index is the net value of the components of nano emulsion and indicates the isotropic nature of formulation. It is the technique for assessing whether formulation is transparent or not also thermodynamic stability analysis of sample.[28]

Method

It is determined by putting sample drop on slide and then comparing with water having RI 1. 33 using refractrometer. If comparison of system's RI is relatable to water's RI, then formulation is transparent.Refractive index was determined by using Refractometer.[28]

7. Conductance

Introduction

Conductometer is used to determine conductance of sample i.e., nanoemulsions.An EC Tester 11+, USA conductance meter was used to test the electrical conductance of the nanoemulsion at 25 degrees. Three runs of this test were conducted to ensure uniformity.[33]

Method

In this method electrodes immersed in emulsion system which is supplied with electric source and lamp. If o/w type emulsion then water conducts current and lamp lights up due to flow of current between electrodes.In case if oil is exterior phase so lamp is dark because emulsion is absent.[28]

8. Viscosity

Introduction

It is key characteristic of nano emulsion. The resistance to flow of fluids is termed as viscosity, or the friction that exists within fluids. The most frequently employed instrument to measure viscosity is Brookfield viscometer.

Method

The viscosity of the produced nanoemulsion was measured using the Brookfield DV-II+ Pro viscometer at 25°C without dilution by taking average of three data points at specific shear rate. After the mixture had been in the beaker for five minutes, spindle readings were taken at 0.5, 1, 2.5, and 5 rpm at maintained temperature and at room temperature for 12 weeks which suggests that the lower the storage temperature, there is increase in viscosity of nanoemulsions. The viscometer's accompanying dial was read and recorded. According to power law model, emulsions exhibit shear thinning behavior under shear rate. It gives three ranges for n(flow behavior index): $n<1$ for shear thinning fluid, $n=1$ for Newtonian fluids and $n>1$ for shear thickening fluids. Emulsions shows less than 1 n value. [33]

Viscosity= mass/volume

The viscosity of nanoemulsions can be determined at various shear speeds. [28]

9. Dye test

Microscopic analysis is done for clear understanding. If o/w emulsion type then it continuously absorbs water soluble dye. Conversely, if w/o emulsion it only uptakes water soluble dye in dispersion phase and the color is not uniform.[28]

10.Creaming test

Following homogenization, 10 milliliters of the nanoemulsions were immediately put into a test tube, firmly sealed, and kept at room temperature (25 ± 2°C) for seven days.

The creaming's stability was determined by visually examining and then calculating the creaming index percent

$$\text{Creaming index percent} = (HL/HE) \times 100\%$$

Where HL is the entire height of the cream layer and HE is the overall height of the emulsions [34].Increased creaming index indicates the presence of emulsion instability, which can be attributed to flocculation, aggregation, coalescence, or high particle size.[30]

11.Creaming stability

Visual observation was used to identify the creaming, which was then quantified using the creaming index %.

Creaming index percent = (cream layer height/total emulsions height) × 100[30]

12.Melting resistance

To evaluate the melting behavior, nanoemulsion containers were taken out of the freezer at a temperature of -20°C in order to measure the meltdown point. The samples were placed on 1.5-1.5 cm by 2.5-2.5 mm rectangular stainless-steel wire mesh screens set atop a funnel that was attached to a graduated cylinder. The nanoemulsionwas then stored in a temperature-controlled room at −25°C. Weighing and recording the water dripping volume every 10 minutes for a maximum of 90 minutes. Both the instant that the first nanoemulsiondrop dripped and the moment it completely melted were timed and recorded. A graduated cylinder was used to record the amount of unmeltednanoemulsion at 30-minute intervals.[30]

13.pH values and total soluble solid content

At −20°C, the total soluble solid content was measured inBrix is the result of triplicate measurements made with a Pocket Pal-1 refractometer. The pH was calculated using the pHmeter by submerging the instrument bulb into 30 mL of each produced formulation[30]

14.Texture Analysis

The nanoemulsion samples were kept in plastic containers at -20°C for a whole day in order to conduct the texture analysis. At room temperature (25 ± 2°C), measurements of firmness, hardness, consistency, cohesiveness, and viscosity index were made using a texture analyzer TAXT2i fitted with a 2-mm-diameter acrylic cylindrical probe. The samples' geometrical centers had a penetration depth of 10 mm, and their penetration rates were 1 mm/s. After being hardened at -30°C, the nanoemulsion was sliced to fit into a tiny cylindrical cup with a diameter of 4.5 cm and a depth of 30 mm. It was then tempered overnight to -15°C in preparation for the analysis. The penetration speed of the probe was 2 mm/s up to a 20 mm distance.[30]

15.Sensory evaluation

The sensory analysis was performed on the nanoemulsion samples. A group of people of different age groups were selected for the sensory analysis Using a 9-point hedonic scale—where 1 represents a very low preference and 9 denotes a very high choicefour nanoemulsionsamples were evaluated for taste, preference, appearance, and consistency.[30]

16.Percentage of transmittance measurement

The % transmittance study was used to assess the clarity of the prepared nanoemulsions. A UV–Vis spectrophotometer was used for this investigation, with deionized water serving as the blank at the drug Lambda max of 210 nm.[35]

17.Dynamic Light Scattering (DLS) Spectrophotometer

The DLS measurement is obtained at 90˚ with a 632 nm neon laser. The instrument determines the size and dispersion of the particles.[36]

18.Droplet size measurement

Using DLS, droplet size measurements were assessed. Prior to measurements and tests, samples were not diluted and were carried out at 25°C. No photosensitizer was used during the experiments in order to Reduce fluorescence interference in the signals from light scattering. [37]

19.Creaming and cracking

Each multiple nanoemulsion (MNE) was sampled in 30 mL and placed in a glass bottle with a screw lid (height 65 mm and inner diameter 25 mm). The container was then allowed to stand at 25 ± 2 °C for a day before being checked for physical changes. The permanent/irreversible division or separation of the internal/dispersed phase (where oil and water are clearly separated) at the top of the emulsion is known as cracking, which is a physical instability. The given Equation was used to calculate the cream layer height (top layer) and the overall emulsion height in the event that the emulsions are divided into cream and serum layers. This allowed for the determination of the percentage of creaming.

$$\text{Creaming (\%)} = 100 \times \text{Height of cream layer/Total height of emulsion}$$

.[36]

20.Entrapment efficiency

To determine the percentage drug encapsulation efficiency, the concentration of unentrapped drug, or free drug, in the formulation was assessed. This concerns since it has an impact on the medication molecule's release characteristics. Equation was used to determine the quantity of drug encapsulated per unit weight of formulation after the entrapped drug was separated from the nanoemulsion formulation.

%EE = (amount of drug added − free (unentrapped) drug)/(amount of drug added) × 100.[36]

21.Thermodynamic stability

Under thermodynamic stability tests, the problem of metastable formulation was resolved. The determination of thermodynamic stability involves three steps:

Centrifugation

The nanoemulsions are subjected to centrifugation at 5000rpm for 30 minutes. The stable preparations do not show any signs of phase separation.Phase separation is observed by centrifuge the sample if it is unstable or metastable emulsion.[37]

Heating cooling cycle

The sample to be assessed is stores at such temperatures for time duration not less than 48 hours which is also exposed to temperature variation between 4° and 45°. Six cycles of temperature exposure is given to each formulation and is assessed for stability.[37]

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Freeze thaw cycle

Formulation is stored over three freeze thaw cycles between 21°and +25° minimum for 48 hours and then its stability is assessed for such nanoemulsions are stored in deep freezer (-20°C) for 24 hours. Then it is removed from freezer and kept at room temperature. The stable nanoemulsions return to their original form in 2-3mins.[37]

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22.Differential Scanning Calorimetry

It is a thermo analytical technique which measures difference in amount of heat needed to increase temperature of sample and reference. Both reference and sample are maintained at same temperature throughout experiment. The sample should have well defined heat capacity over the range of temperature. This technique is employed to detect phase transitions as melting of

crystalline agents and analyze proportion of solid fat or ice crystals in emulsion. It is also used to detect crystallization temperature of mixture of surfactants.[25]

23. FTIR

It is based on infrared radiations that are absorbed by sample. It gives spectrum that represent molecular absorption and transmission forming molecular finger print of sample. This fingerprint represents characteristic absorption peaks corresponding to frequencies of vibration between atom of material. The size of peaks in spectrum is direct indication of amount of material in sample. Advantage of FTIR is to determine amount of component in mixture and to determine quality and consistency of sample. It gives accurate and reproducible measurements.[25]

24.NMR (Nuclear Magnet Resonance)

This analytical tool studies compounds in liquid or solid states, providing molecular structure information, stereochemistry, and repeating distances up to 150nm. It's nondestructive, requires minimal sample preparation, and offers complementary techniques.[25, 38]

25.In vitro dissolution profile

USP Apparatus type-II

A dissolution apparatus type II was used for a drug release investigation. 900 milliliters of pH 1.2 simulated gastric fluid served as the dissolving medium. Every nanoemulsion formulation has gone through this investigation at various pH levels by being placed in a dialysis membrane bag and replaced with fresh medium. At certain intervals, a 5 mL sample was taken and replaced with new media. Each sample was examined to determine drug concentration using a UV-Vis spectrophotometer set to analyze at lambda max of 210nm after being filtered with a syringe filter of 0.45 µm. Then samples are analyzed in HPLC or by other methods for determining release behaviour which is then compared with standards.[35]

Franz cell apparatus method

The Franz cell device with a diffusion area of 1.79 cm2 and a receiver chamber volume of 16 ml was used for in vitro drug release. A synthetic cellulose acetate membrane (Merck, Brazil) was used, which had been previously moistened. The donor and receiver compartments were then separated by the membrane. To maintain sink conditions, the receiver chamber was filled with a

physiological solution containing 1% -cyclodextrin as a solubilizer (maximum MS solubility = 2.13 mg/ml). A Peltier-Type Temperature Control Equipment was used to keep the receptor compartment at 32 °C by employing an external thermal bath. Throughout the experiment, continuous stirring was maintained. Bubbles were avoided by sonicating the receiver solution before to the experiment.

On top of the membrane, the equivalent of 10 mg of formulation was deposited. The donor compartment was secured to the receiving compartment, which was then sealed with Parafilm®. Aliquots of 500 l were collected after 30 minutes, 1, 2, 3, 4, 6, 7, 8, and 24 hours. An equivalent volume of new medium was poured to maintain the washbasin condition. A validated HPLC method was used to determine the concentration of MS in each sample.[36]

Membrane diffusion method

Drug release studies was carried at temperature 32°C by using (standard regenerated cellulose and Spectra). For Experiment of release take 5g of Formulation along with the receptor solution filled in dialysis chamber for 24 hours. By using UV-Visible Spectroscopy the concentration of drug in receptor solution was analyzed by wavelength in 350nm. Surfactant was added in solution to increase solubility in receptor solution. The receptor solution consist of 1.5% w/w polysorbate buffer at pH (7.4). [39]

Comment [U6]: Justify

26.Drug release kinetics

Using the Microsoft Excel® add-in DDSolver, the dissolving data were fitted to different kinetics models, including Higuchi, Korsmeyer-Peppas, Hixson-Crowell, Hopfenberg, Peppas-Sahlin, Weibull, and Makoid-Banakar [33]. The best appropriate mathematical model for each formulation was interpreted by analysing the adjusted coefficient R2 (Rsqr_adj) and the residual standard deviation (MSE_root).[40]

Release kinetic studies were performed to determine the efficacy of active substances. There are number of kinetics models which describe the release of drug from above models.[39]

Zero order model

Drug concentration and release rate are independent of one another in zero order release kinetics, which describes continuous drug release from the delivery device. The following equation can be used to describe the zero-order release kinetics.

$$Qt = Q0 + K0 \cdot t \quad (1)$$

Qo = First drug released in device, Q= Constant (zero order release), and Q= Drug released quantity in time t.If zero order release kinetics are being examined, the cumulative drug release (Q) Vs time (t) plot forms a straight line with an intercept at Qo and a slope of Ko.[41].

First order model

Drug release from a system where drug concentration depends on release rate is described by this release kinetics model. The following equation can be used to characterize the medication release pattern.

$$dC/dt = -K1C$$

Another way to define it is LogC=logCo-K1t/2.303, where C is the quantity of medication released in t time, Co is the amount of drug released initially in the device, and K1 is the constant (first order release).If a first order drug release pattern is seen, the log of cumulative drug remaining versus time t plot forms a straight line with an intercept of log Co at t= 0 and a slope of K1/2.303.[41]

Higuchi model

Higuchi devised this model in 1961 and 1963 for the investigation of drug release from intractable matrices using Fickian diffusion. [41] This approach was also suggested for the investigation of medication release from low- and water-soluble substances. [42]. The Higuchi model uses the square root of a time-dependent mechanism using a solid dosage form to explain drug release.The following formula can be used to describe the Higuchi model.

$$Mt/M\infty = KH * t½$$

The cumulative drug release quantity in time t and infinite time, respectively, is represented by Mt and M∞ here.KH stands for Higuchi constant in drug release.Following the Higuchi model for drug release will result in a straight line with a slope of KH on the Mt/M∞ vs. time t plot.Diffusion process defines drug release, and Higuchi model is based on Ficks law of diffusion.The Higuchi model was originally developed for planar systems, but it was later expanded to include a variety of porous and geometric systems. [43]

Korsemeyer Peppas Model:

The fractional release of the medication as a function of time is explained by this model.It is represented by the following equation and adequately describes how drugs are released from polymeric systems.

$$Mt/M\infty = Kt\hat{}n \quad \text{or} \quad Log\ (Mt/M\infty) = \log K + n\ \log\ t$$

Where K is the rate constant, n is the exponent of diffusion, which defines the drug release mechanism, and Mt/M∞ describes the proportion of drug release in time t.Plotting log (Mt/M∞), or log cumulative drug release percentage vs. log time, will show a straight line with a slope of n if this model is followed. [39, 41]

APPLICATIONS

Comment [U7]: Include current research activities and marketed products details

1. Nanoemulsions in drug delivery

Nanoemulsions are utilized in various drug delivery methods, including topical, ocular, intravenous,intamuscular,internasal, and oral delivery. They utilize their lipophilic nature to dissolve water-insoluble drugs and their tunable charge and rheology to create aqueous solutions. Nanoemulsions also offer advantages for hydrophobic drugs, and are used as ultrasound imaging agents.[44]

2. Oral Delivery

Lipids can be used as nanoemulsions to increase drug absorption in the GIT, particularly protein drugs, by loading them inside lipids, thereby enhancing the overall absorption process[45]

3. Topical delivery

Enhancing the permeation of drugs for topical application is challenging due to poor dispersibility and skin irritant effects. Nanoemulsions, such as soybean lecithin, tween, and poloxamer, offer a combination of penetration enhancement and concentration gradient.

4. Intravenous delivery

Parenteral nanoemulsions deliver drugs with lower bioavailability and narrow therapeutic indices, converting into stealth nanoemulsions by coating or attaching hydrophilic moiety, enhancing permeability and retention for tumor targeting.

5. Use in cosmetics

Newer materials (NEs) are increasingly important for controlled cosmetic delivery and optimized dispersion of active ingredients in skin layers. They are suitable for transporting lipophilic

compounds and support skin penetration, increasing active ingredient concentration. NEs also have bioactive effects, reducing trans-epidermal water loss and strengthening skin barrier function. They are acceptable in cosmetics due to their lack of creaming, sedimentation, flocculation, and coalescence[10]. TRI-K Industries and Kemira have developed a new nano-based gel, KemiraNanoGel, to improve the effectiveness of skincare products. The unique NE Carrier system creates submicron emulsions from an oil-in-water concentrate, minimizing trans epidermal water loss and enhancing skin production. This technology is particularly useful in sun care, moisturizing, and anti-aging creams, and provides a good skin feel.

6. Nanoemulsion in cell culture technology

Cell cultures are used for in vitro assays and producing biological compounds. Oil-soluble substances have been difficult to absorb by cells. New encapsulated substances (NEs) are a new method for delivering oil-soluble substances to mammalian cell cultures. These transparent, phospholipid-stabilized NEs have high bioavailability, improving cell growth and vitality, and allowing for toxicity studies of oil-soluble drugs in cell cultures.

7. Nanoemulsion as antimicrobial preparation

NEs are increasingly important for controlled cosmetic delivery and optimized dispersion of active ingredients in skin layers. They support skin penetration, increase active ingredient concentration, and have a small droplet size. NEs also have bioactive effects, reducing trans-epidermal water loss and avoiding creaming, sedimentation, flocculation, and coalescence compared to macroemulsions[10].TRI-K Industries and Kemira have developed a new nano-based gel, KemiraNanoGel, to improve the effectiveness of skincare products. The unique NE Carrier system creates submicron emulsions from an oil-in-water concentrate, minimizing water loss and enhancing skin production. This technology is particularly useful in sun care, moisturizing, and anti-aging creams.

8. Nanoemulsion to improve the per-oral delivery of poorly soluble drugs

Nanoemulsion has wide application in improving solubility of poorly soluble drugs.BCS class II and IV drugs, which are poorly soluble in water, face challenges in conventional dosage forms. Nanoemulsions offer a solution for improved solubility and therapeutic efficacy.[46]

9. Nanoemulsion in transdermal drug delivery system

Nanoemulsion technology has been widely utilized as transdermal drug delivery system.Nanomaterials' small size allows them to connect more substances due to their large surface area and easy transport, and their surface drainage allows them to accumulate at skin level.[47]

10. Nanoemulsion in targeted Drug delivery system

As a targeted drug delivery system nanoemulsion has been widely utilized in cancer therapy.Nanoemulsions are increasingly used in cancer diagnostics, imaging, and therapy due to their efficient solubilization, biocompatibility, high stability, and ability to accumulate in pathological areas with defective vasculatures, overcoming anatomical and physiological barriers.[48]

CONCLUSION

This study explores various nanoemulsion formulating techniques, preparation, characterization, release studies, and kinetic modelling. This document discusses various high- and low-energy techniques such as high-pressure homogenization, ultrasonication, microfluidization, phase inversion by temperature, solvent displacement method, and self-emulsifying nanoemulsion, respectively, that are considered the best methods for formulating nanoemulsion. All nanoemulsion formulations are generally considered effective, safe, and have improved bioavailability. Nanoemulsions offer advantages in drug delivery, masking oily liquids' unpleasant taste, and protecting drugs from hydrolysis and oxidation, making them popular for targeted anticancer, photosensitivity, and therapeutic agents. Moreover, various applications of nanoemulsion are also being discussed, including the use of nanomeulsion in drug delivery systems like oral, topical, intravenous, intramuscular, intranasal, pulmonary, and ocular. Nanoemulsion also has broad applications in cosmetics, cell culture technology, and antimicrobial preparation to improve the per-oral delivery of poorly soluble drugs in transdermal drug delivery systems.

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