Optimization, Characterization of Artemether and Lumefantrine Nanosuspension for Solubility and Bioavailability Enhancement

Sanjana Gaikwad[1]*, Madhukar Tajne[2]
University Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur (M.S.), India.

Abstract: Present study describes the formulation of Nano suspension System of Antimalarial Drugs Artemether and Lumefantrine with lipids and surfactants which enchanced the solubility and bioavailability. The poorly soluble drugs, were considered to be fit for nanosuspension to improve its solubility. The study intended to preparation of nano suspension by means ofZirconium beads milling method and furtherLyophillisation. Artemether Lumefantrine consists of PVP and SLS. Nanopowders were further characterized for particle size, Zeta Potential and Drug contentwas to be 732.9 nm, -0.2 mV and 99.25 %. During the screening processes, the formulation showed a smaller particle size and stability during lyophilization.*In vitro*studies reported to be90.34±0.21% for Artemether and 92.03±0.72%for Lumefantrine. The *in vitro*percent drug release of Artemether and Lumefantrine from Nanopowder found to be higher as compared to marketed formulation (Lumerax®) and pure drugs. The Drug Excipient compatibility studies carried by FT-IR and XRD depicted that there was no interaction between drugs and excipients.Moreover it was suggested that stabilizersPVP and SLS were appreciated excipients on the Nanopowder platform and exhibited the abilities of size-reduction and stability-maintaining on freeze-drying

Keywords: Anti-malarials, Nanosuspension, Freeze drying, enhanced solubility, dissolution, Oral bioavailability.

INTRODUCTION

Figure 1: Zirconia Beads

Wet milling

A practical and well-established method to enhance the absorption of these active pharmaceutical ingredients (APIs) is particle size reduction, especially for those molecules having a dissolution rate limited bioavailability [1]. Particle size reduction can be regarded as a nonspecific formulation approach to enhance the bioavailability of poorly soluble compounds because it can be applied for almost every poorly soluble compound independently from its solid state or other physico-chemical properties [2]. By reducing the particle size of the drugs, one can achieve a significant increase in surface area. According to the Noyes–Whitney equation, an increase in surface area results in faster rates of dissolution [3]. Simultaneously, according to the Ostwald–Freundlich equation, the saturation concentration at the surface of small particles, especially in the lower nanometer range, is higher than the saturation concentration at the surface of large particles [4]. Often times drug products containing nanosized APIs possess also reduced or even eliminated food effects. Drug nanocrystals can be produced by employing various particle size reduction technologies. Depending on the production method, they can be classified as bottom-up and top-down.

Top-down processes are by far the most important industrial relevant particle size reduction technologies. Typical top-down processes are high pressure homogenization and wet ball milling [5]. Using these technologies, one starts with a micronized drug powder, which is suspended into an aqueous/non-aqueous dispersion medium containing surfactants or polymeric stabilizers [6]. Micronized API is recommended in order to shorten the required time for the diminution process and to prevent a clogging of the machines. Therefore, in general, jet-milled drug powders have to be used [2]. The suspension is then, for example, passed through a ball mill or a high pressure homogenizer [7-9]. The larger drug particles are broken down to very small drug nanocrystals. In contrast to the bottom-up technologies, almost any poorly soluble drug can be processed, also those being poorly soluble in aqueous and simultaneously in non-aqueous media [10]. However, depending on the physico-chemical properties of the drug and the processing parameters, different durations of the particle size reduction processes are needed in order to obtain a nanosuspension. From industrial and economical point of view it is highly desirable to minimize the milling times or the number of homogenization cycles [2].

To overcome the limitations of the conventional particle size reduction technologies for poorly soluble drugs, new combinational methods have been developed for the production of ultrafine suspensions. Combinative technologies are a relatively new approach to improve the particle size reduction effectiveness. In general, they can be described as a combination of a bottom-up process followed by a top-down technology [11]. Examples from bottom-up technologies are spray drying and freeze drying. Spray drying has also been widely used as a technique to improve the dissolution rate of drugs. However, it is not always possible to find a suitable solvent for the spray drying process. Another limitation is that spray drying in general is not the first choice for thermolabile compounds because the spray drying process could lead to elevated temperatures [12]. An alternative bottom-up process is freeze drying. It can also be coupled with a top-down step to produce ultrafine drug nanoparticles, for example, high pressure homogenization [13]. The freeze drying process involves freezing a solution. The frozen solution is then exposed to a very low pressure, at which the ice formed is eliminated by sublimation. The majority of the pharmaceutical products using this technology are lyophilized from aqueous solutions. With the increasing problem of poorly water-soluble API's, the freeze drying with organic solvents systems has become an interesting strategy for the formulation of problematic API's. Lyophilization or freeze drying is a promising technique to produce pharmaceutical powders with enhanced dissolution rate, although the freeze drying process is relatively slow [4]. Therefore, freeze drying is regarded as costly unit operation. Consequently, the production costs for the combinative method will be also higher compared to particle size reduction alone. However, the significantly improved particle size reduction effectiveness, both in terms of process time and minimal achievable particle size, could justify its application especially in case of expensive, labile compounds. The freeze drying technology also modifies the structure of API powders, which is interesting when using a secondary top-down step to nanosize a suitable breakable material [13]. The work described here is a combination of a non aqueous freeze drying step (bottom-up) followed by wet ball milling or high pressure homogenization (top-down) to produce fine drug nanocrystals. A schematic description of this novel combinative technology is mentioned in Figure 1.4.

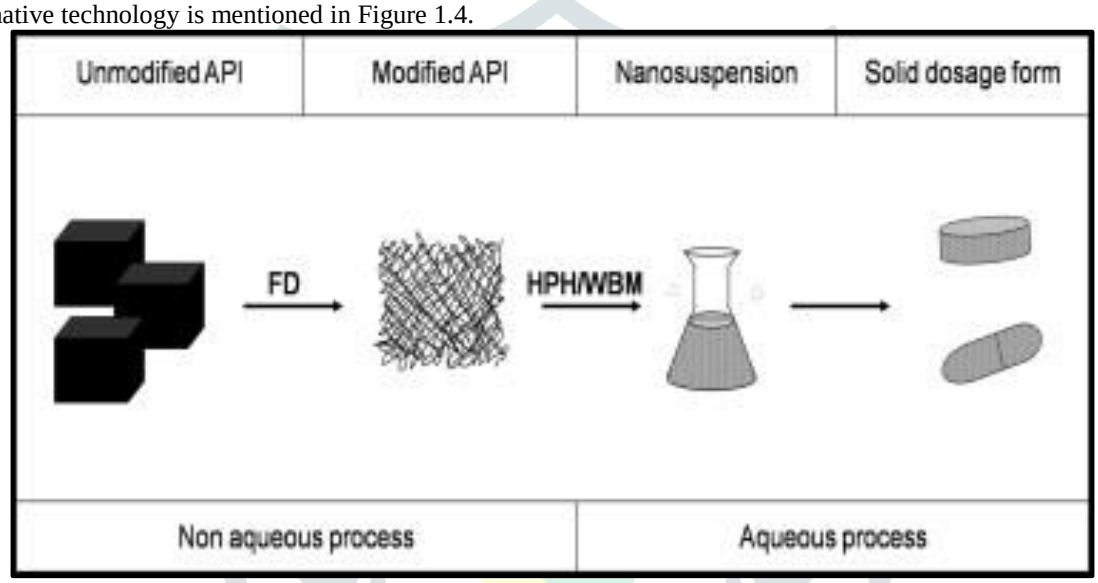

Figure 2: Schematic Description of the combinative H 96 Technology for Nanoparticle Production. FD: freeze drying, HPH: high pressure homogenization, WBM: wet ball milling.

The investigations presented are concentrated on the systematic research of parameters influencing the reduction effectiveness and on the comparison of two top-down technologies. A poorly water soluble (BCS class II) model compound (Glibenclamide) was used to determine the optimal process parameters for the development of a better and faster method for the particle size reduction of poorly soluble drugs [13].

Nanosizing refers to the reduction of the active pharmaceutical ingredient (API) particle size down to the sub-micron range. While reduction of particle size has been employed in pharmaceutical industry for several decades, recent advances in milling technology and the understanding of such colloidal systems have enabled the production of API particles of 100–200 nm size in a reproducible manner. The sub-micron particles are stabilized with surfactants or polymers in nanosuspension which can be further processed into standard dosage forms, such as capsules or tablets, suitable for oral administration. These nano formulations offer increased dissolution rates for drug [14-17].

Apart from the techniques afore mentioned, another strategy employed to improve solubility and ultimately, bioavailability of poorly water-soluble drugs is milling. The terms milling, size reduction, comminution, grinding and pulverization are often used interchangeably. Milling is a unit operation where mechanical energy is applied to physically break down coarse particles to finer ones and hence, is regarded as a "top down" approach in the production of fine particles [18]. As virtually every drug can be comminuted to fine particles regardless of its solubility in aqueous or non-aqueous solvents, the "top down" approach has wider commercial and industrial applications than the "bottom-up" approach (e.g. precipitation) where fine particles are constructed from their dissolved molecular state and suitable solvents/anti-solvents of the drug need to be selected. Traditionally, milling is carried out to facilitate the extraction of crude drugs or to improve their bulk processing properties. Cutter mills, roller mills, pestle and mortars and runner mills may be employed for this purpose. In these milling operations, the dried crude drug may be cut by sharp blades (cutter mill), impacted by hammers or crushed/compressed by the application of pressure (rollermill, pestle and mortar). As a limited amount of energy is imparted, the milled particles remain relatively coarse. Technological advancements in milling equipment now enable the production of ultrafine drug particles down to the micron or even sub-micron dimensions. Griseofulvin, an anti-fungal drug, represents one of the pioneering examples of drugs where solubility and absorption were enhanced by milling. Milling of Carbamazepine was found to be more effective in enhancing drug dissolution than formulating the drug as a solid dispersion due to polymorphic transformation of the drug (from the b to a form) in the solid dispersion system [19].

Mechanism of milling

Mechanisms by which milling improves drug dissolution and solubility milled products possess specific physical attributes that contribute to improved drug dissolution and solubility. Milling reduces the size and alters the size distribution of the drug particles. These properties may be measured by light scattering techniques such as photon correlation spectroscopy (5 mm down to 0.001 mm) and laser diffraction (0.05 mm-2000 mm), respectively [20]. By virtue of their smaller size, milled particles possess larger specific surface area compared to their unmilled counterparts. Based on the Noyes Whitney equation, it is likely to increase the dissolution rate of the milled drug particles if the particles can also be adequately wetted. Milled particles possess higher surface free energies and this, coupled with their thinner diffusion boundary layers [21]further enhance the dissolution rate of the milled drug substance. Apart from size, milling also alters the surface roughness and shape of particles. It has been demonstrated in many studies on the development of inhalable dry powder formulations. It has been shown that the surface properties of milled particles can affect wetting [22]and dissolution behavior [23]. Furthermore, milled particles are rarely isometric or spherical in shape. Compared to particle size, considerably lesser attention has been devoted to the impact of particle shape [24-28]on drug dissolution, solubility and bioavailability although early studies have demonstrated that when particles are platelet-like or possess needle shapes, the shape factors of particles are closely related to their dissolution rates and profiles [29-33].

Particle shape may be determined by image analysis techniques, laser diffraction [34], scanning electron microscopy, transmission electron microscopy and atomic force microscopy. In a milling operation, particle size reduction ceases at a practical limit [35]beyond which the material becomes progressively difficult to comminute even when milling time is prolonged. When particle size reduction has reached a critical threshold, the continued transfer of mechanical energy from the mill to the drug substance leads to the accumulation of defects on the drug crystal and disordering of the crystal structure, eventually bringing about the disappearance of the order in the positions of atoms or molecules in the crystal. These defects may manifest throughout the entire crystal resulting in complete amorphization of the drug or be restricted to the crystal surfaces in which case a thin, amorphous (disordered) layer may be formed around a crystalline (ordered) core. Under these circumstances, the drug is said to be “mechano chemically-transformed” or “activated” by the milling process. Drug amorphization as a result of milling improves the aqueous solubility and dissolution characteristics of the drug. It may also confer additional benefits such as improved compressibility. However, the disadvantage of these solid state transformations is that amorphous regions or crystal defects created may be thermodynamically unstable, leading to amorphous-crystalline inter-conversions of the drug during storage, alteration of particle size distribution, specific surface area, chemical and physical reactivity, dissolution or overall performance of the drug product. An approximate measure of the extent of milling-induced drug activation may be obtained experimentally by determining the amorphous content or residual crystallinity before and after milling the drug substance using standard solid state characterization tools such as X-ray powder diffraction (XRPD), differential scanning calorimetry. As the information obtained from these different techniques complement each other, a combination of techniques is often desired to fully elucidate the solid-state condition of the milled drug substance [36].

The micro or nanoparticles produced from milling possess a large surface/interfacial area, increased free energy and decreased thermodynamic stability. These factors promote particle agglomeration. Mechano chemically-activated particle surfaces and amorphous regions generated during milling also increase the surface free energy of the particles, favoring agglomeration. In practice, it has been suggested that particle agglomeration arising from Vander Waals' and other forces (e.g. electrostatic forces) become significant at particles sizes of about 30 mm and below. Fine, hydrophobic drug particles less than 5 mm size are known to be exceptionally prone to agglomeration and this is attributed to the inter-particulate cohesive forces between them. Hence, when milling is prolonged, particle agglomeration may supersede particle fracture and this severely reduces the efficiency of the mill overtime. Agglomeration occurring during or after milling reduces the effective surface area of the drug particles, with their resultant dissolution rate and bioavailability, being comparable or even less than their untreated counterparts. In most cases, drugs are co-milled together with certain adjuvants to minimize the conditions promoting agglomeration. These adjuvants are inert, non-toxic pharmaceutical excipients that function as a carrier and/or stabilizer of the drug in the milled product. Typically, the excipient employed is hydrophilic in nature and notable examples are hydrophilic polymers such as Polyvinylpyrrolidone, Cellulose ethers, Polyethylene glycol, Polyvinyl alcohol or Poloxamers; surfactants, ionic or non-ionic; inorganic materials like Magnesium aluminometasilicate and Cyclodextrins. By conferring hydrophilicity to the hydrophobic drug particle surfaces, the added excipients also enhances the wet ability, solubility and bioavailability of the poorly water-soluble drug. The efficiency of a particular stabilizer depends on its potential for interaction with the drug compound. Generally, milling may be conducted with the drug in its dry state (dry milling) or suspended in a liquid medium (wet milling). In dry milling, the mechanical energy imparted fosters drug-excipient interactions via Vander Waals forces or hydrogen bonding. The resultant drug-excipient composite particles are often stable, exhibit low tendencies to agglomerate and retain the activated status of the drug [37-41].

Drug nanoparticles are most commonly produced by wet milling. As the name suggests, wet milling involves size reduction of drug particles suspended in a liquid medium that may be aqueous or non-aqueous in nature. Wet milling is particularly suited for potent drugs and drugs which possess high residual moisture contents (>50% moisture) because dry milling may be problematic for drugs of this nature. In wet milling, a drug nanosuspension is produced as the end product although for improved product stability (minimization of Ostwald ripening and possible hydrolytic degradation of drug), patient convenience and the drive towards green or sustainable manufacturing processes, the nanosuspension may subsequently be transformed into a solid dosage form.

Excipients /Milling adjuvants

In wet milling, the addition of surfactants (e.g. Sodium Lauryl Sulfate and Polysorbate 80) and polymers (e.g. Hydroxypropylmethyl Cellulose, Hydroxypropyl Cellulose, Polyvinylpyrrolidone and Poloxamer), singly or in combination, helps to minimize the agglomeration of suspended particles via electrostatic and steric mechanisms. Steric stabilization is achieved when long chain polymers are adsorbed onto the surfaces of the drug particles, forming a physical barrier that prevents the close approach of the particles. The chain length, molecular weight, hydrophobicity, concentration, shape and surface energy of the polymer will influence the efficiency of adsorption. The method of adding the polymer (periodic additions or additional the start of milling process) also affected its stabilizing properties [42-46].

Electrostatic stabilization is achieved when charged polymers or ionic surfactants become adsorbed on the surfaces of the drug particles and lower their apparent charge. Apart from preventing agglomeration, these stabilizing molecules may also aid in preventing crystal growth (Ostwald ripening) that could adversely alter the dissolution and bioavailability of the drug suspension after storage. Hydroxypropylmethyl Cellulose (3 cps) was found to stabilize and minimize crystal growth in a nanosuspension of an unidentified drug compound NSV-102 (Novartis Pharma) produced by media milling, an example of wet milling. This was attributed to improved surface coverage owing to its stronger interaction with the drug in comparison with other stabilizers, Pluronic F-68, Pluronic F-127, Sodium Lauryl Sulfate and Polyvinylpyrrolidone K-30, investigated. A screening study of polymers, copolymers, and surfactants has revealed that the stabilizing performance of surfactants to be the best followed by linear synthetic polymers and semisynthetic polymers [61]47.

Milled drug particles are intermediates in the production of Pharmaceutical Dosage Forms. Oftentimes, they are cohesive and exhibit poor flow properties, largely due to their higher surface energies compared to their coarser counterparts. To alleviate this problem, inert pharmaceutical excipients or fillers, e.g. calcium phosphate, lactose, Mannitol and other sugars etc., are often added and mixed with the milled drug particles to improve powder flow. Alternatively, the drug particles may be granulated with these fillers to form granules which typically exhibit improved flow properties and content uniformities than the corresponding physical mixtures. Apart from improving flow during manufacturing, these fillers may also serve other functions e.g. modifying drug release, enhancing drug stability and dissolution as well as taste-masking.

Achieving the desired size, shape and activation of drug particles in a milling process often requires extensive optimization of a multitude of process and material-related variables. In terms of processing, a prudent selection of the type of milling equipment is required, followed by the adjustment of the conditions of milling such as the duration of milling, material feed rate and other operational or equipment parameters.

Occasionally, a combination of milling techniques may be necessary to achieve the desired outcomes. When such combination techniques are used, numerous process related variables need to be adjusted and fine-tuned as it allows the unique advantages of each milling technique to be synergistically combined for the desired outcome. Suitable and compatible adjuvants have to be selected to minimize agglomeration, improve wetting, stability and resultant solubility of the milled drug particles. The background from literature on the common milling techniques employed for size reduction is mentioned in literature and are mentioned in Table 1.

II.MATERIAL AND METHODS

Artemether was procured as a gift sample by Ipca Laboratories Ltd., Mumbai, India. Lumefantrine was provided as a gift sample by Zim Laboratories, Nagpur, India

Method

Preparation of Nanosuspension of Artemether and Lumefantrine by Wet Milling Technique

Two types of nanosuspension of Artemether and Lumefantrine were prepared.

1) Nano suspension were prepared by

1) Wet milling
2) Drying process using Lyophilzation

2) Characterization

i) Appearance
ii) Particle size
iii) Chemical Degradation
iv) Drug content
v) Zeta Potential
II) Drying (wet milled dispersion /suspension)
1) Organoleptic properties
2) Particle size

Preparation of suspension wet milling

A laboratory scale media milling apparatus was used for the preparation of nanosuspension. The method is simple, reproducible, fast, economic and one of the easiest procedures for the preparation. Briefly, Artemether and Lumefantrine were dispersed uniformly in an aqueous dispersion medium containing dissolved dispersing agent in different concentrations using a magnetic stirrer operating at 500 rpm. The milling chamber was charged with the milling or grinding media comprising of Zirconium beads. The temperature of suspension was kept almost at ambient during milling due to its mild grinding. The compositions of mixed slurries used for formulation of nanosuspension of Artemether and Lumefantrine is mentioned in Table .1

Table 1: Compositions of Mixed Slurries Used for Formulation of Nanosuspension of Artemether and Lumefantrine

Sr.No.	Formulation code	Dispersing agent	Concentration of dispersing agent (%w/v)
1	S1	PVP	0.5
2	S2	HPC + SLS	0.5+0.1
3	S3	HPMC + SLS	0.5+0.1
4	S4	PVA + SLS	0.5+0.1
5	S5	PVP+ SLS	0.5+0.1
6	S6	PVP + Tween 80	0.5+0.1

Characterization of Nanosuspension

Organoleptic properties

Nano suspension was visually inspected for color, odor and texture.

Particle Size Measurement

The particle size measurement of each drug slurry was done after preparation of suspension. About 0.2 ml suspension was dissolved in 100 ml of distilled water and the median particle size was calculated by particle size analyzer.

Drug content

The percent drug content of Artemether and Lumefantrine in nanosuspension was estimated by the dissolving appropriate quantity equivalent to 120 mg Artemether and 20 mg Lumefantrine in mobile phase and was analyzed by HPLC. Uniform content was observed for the formulations which areas per the IP specification (90-110%).

Chemical degradation

To assess the chemical degradation post-wet milling in suspension HPLC was used. Pre-milled and milled samples were dissolved in mobile phase. To determine the degradation, the percentage area of drug peak was compared against total area of all the peaks in the chromatogram. Any decrease in the percentage area of the drug peak in the premilled or milled sample was considered as degradation [48].

Preparation of Nanopowder

The milled slurries containing drug nanoparticles were readily lyophilized in lyophilizer to obtain Nanopowders, where the nanosuspension was ultra centrifuged for 2 h to separate the solid component from aqueous medium. Then, the paste-like precipitate was recovered and the supernatant was removed and the precipitate was freeze dried for 48 h at temperature of about -20°C and vacuum maintained at 0.120 mBar.

Characterization of Nano powder

Organoleptic properties

Lyophilized Nanopowders were visually inspected for color, odor and texture.

Particle size analysis

The average diameter lyophilized nanosuspension was determined using Dynamic Light Scattering (DLS) technique at 25°C. The Nanopowder was dispersed in distilled water and sonicated to form nanosuspension and was then analyzed for determination of particle size.

Zeta potential measurement

The suspension was diluted with a ratio of 1:2500 (v/v) with distilled water and mixed with magnetic stirrer. Zeta potential for microemulsion was determined using Nanosizer [49].

Characterization of optimized formulation

Differential Scanning Calorimetry

The physical state of drugs and formulation was characterized by Differential Scanning Calorimetry. The sample was placed in standard Aluminium pan, and dry Nitrogen was used as effluent gas. The sample was scanned at speed of 10^0C/min and heat flow from 0^0C to 80^0C. Differential Scanning Calorimetry was performed to study the thermal behaviour of drug.

Infrared Spectroscopy

The baseline correction was carried out using dried Potassium bromide disc and then the spectrum of dried mixture of drug/formulations and Potassium bromide was recorded by placing the compressed disc in the light path.

Scanning Electron Microscopy

The surface morphology of drugs and formulation were determined using Analytical Electron Microscope. The sample was lightly sprinkled on double adhesive tape stuck on Aluminium stub. The stubs were then coated with Platinum to a thickness of above 10^oA under an Argon atmosphere using a Gold sputter module under a high vacuum evaporator. Afterwards, the stub containing coated sample was placed in Scanning Electron Microscope chamber.

X-Ray Diffractometry

X-ray scattering measurements on drugs and formulation were carried out at a voltage of 40 kV and current of 25 mA using Cr as a tube anode material. The solid were exposed to Cu –K radiation angles from 10°- 70°[50].

III.RESULTS AND DISCUSSION

Nanosuspension Preparation

The different formulation batches were prepared and given in Table 1.In preliminary manufacturing trials of the formulation the loading volume of the zirconia beads were kept approximately equal to that of dispersing solution to enhance the milling power. It has been reported that using smaller beads such as 0.03 mm for wet-milling of inorganic substrates made smaller particles having a sharper particle size distribution profile compared to using larger size of beads. The smaller particles having a sharper particle size distribution profile would provide faster dissolution and avoid a particle growth by Ostwald ripening [49].

Characteristics of nano suspension prepared by wet milling

Particle size

The particle size of various formulations of nano-suspension is given is Table 2 and shown in Figure 3-8.

Table 2 Particle size of Nano suspension

Sr. No.	Formulation code	Particle size(nm)
1	S1	1042.9
2	S2	1016.1
3	S3	1329.0
4	S4	614.5
5	S5	526.1
6	S6	619.9

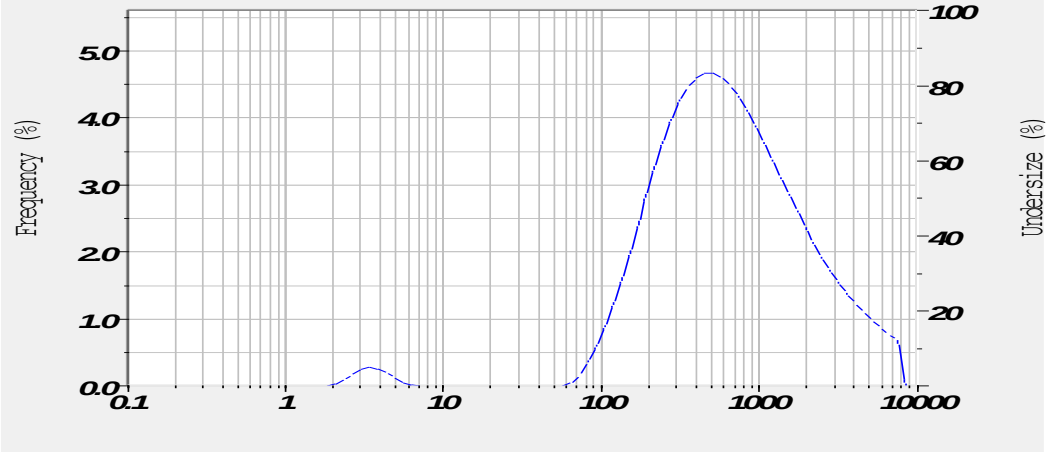

Figure 3: Particle size Analysis of Formulation S1

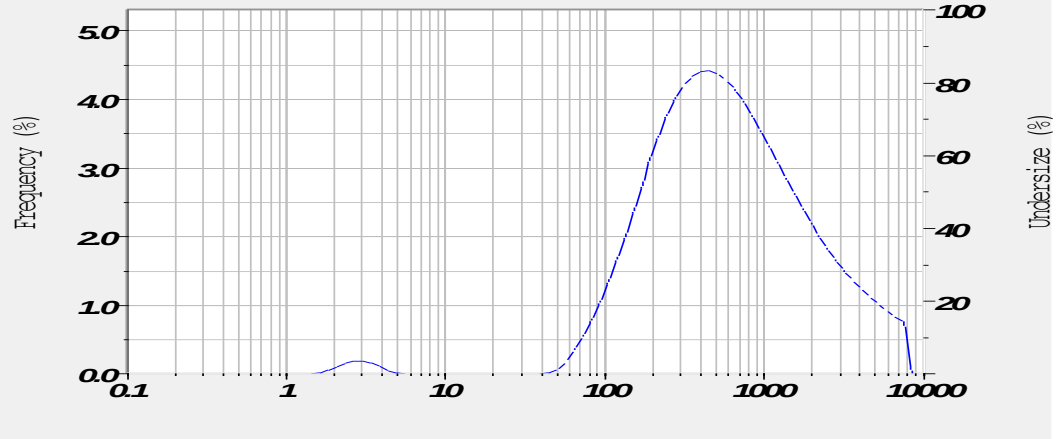

Figure 4: Particle size Analysis of Formulation S2

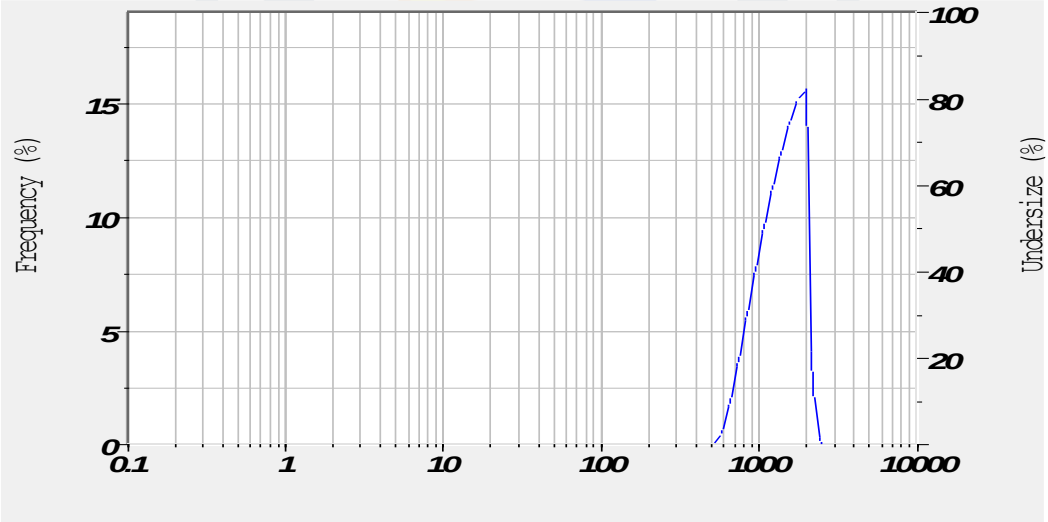

Figure 5: Particle size Analysis of Formulation S3

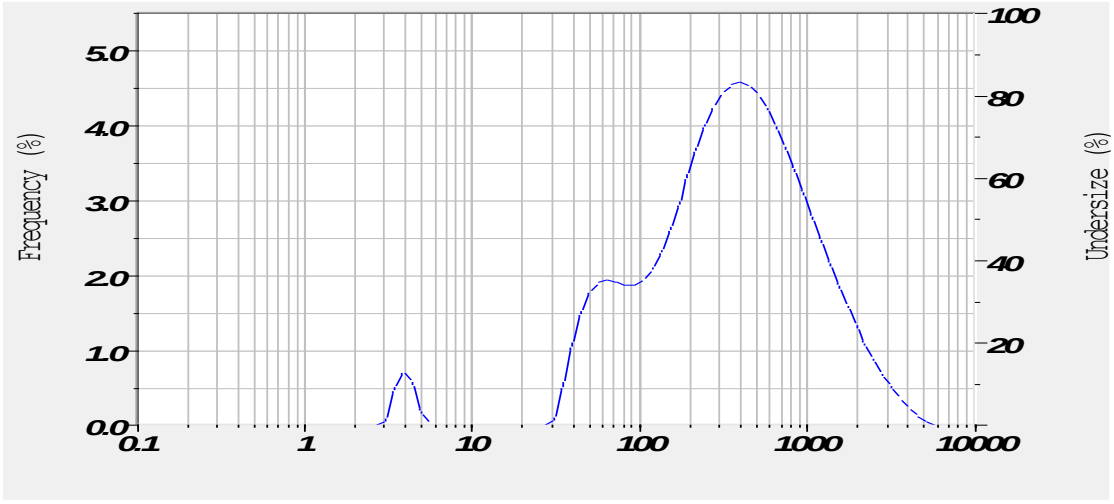

Figure 6: Particle size Analysis of Formulation S4

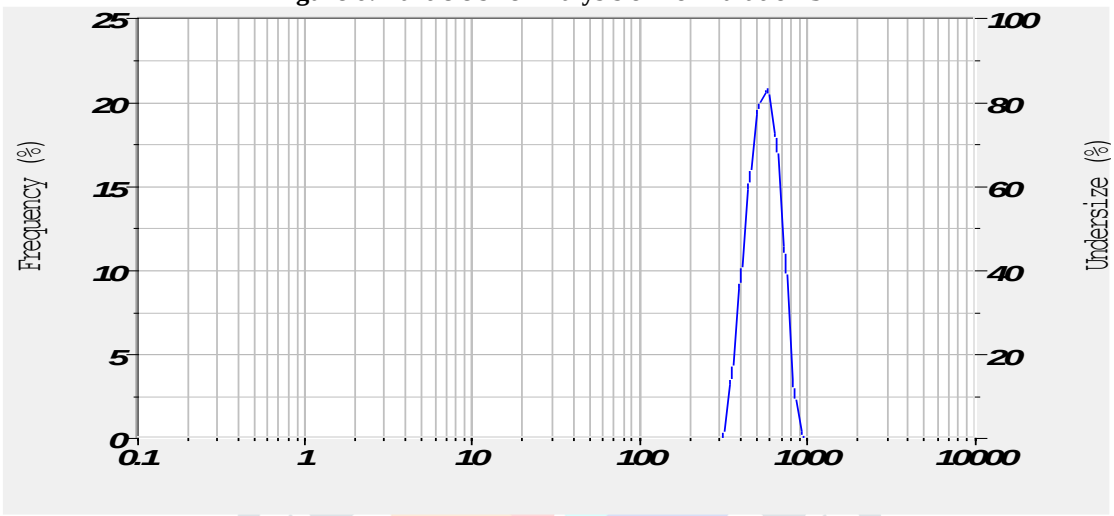

Figure 7: Particle size Analysis of Formulation S5

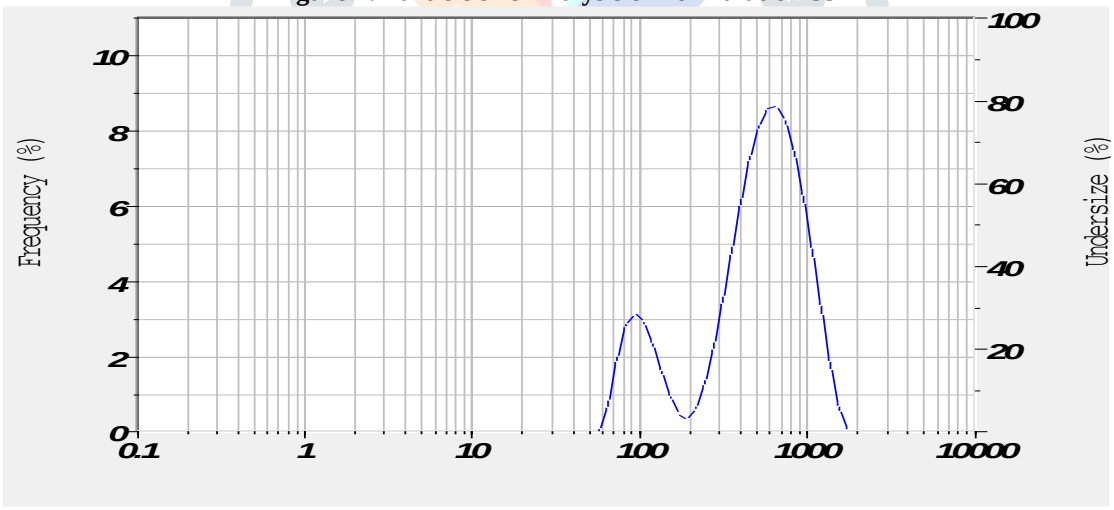

Figure 8: Particle size Analysis of Formulation S6

The temperature of suspension was kept almost at ambient during milling due to its mild grinding. These results indicated that the specific milling machine with high power is not always required to prepare nanosuspensions if the dispersing agents in the medium are appropriately formulated. It was found that batch S5 containing 0.5% PVP+ 0.1% SLS had lowest particle size.

Drug content

The percent drug content of Artemether and Lumefantrine in nanosuspension was estimated by the dissolving appropriate quantity of Artemether and Lumefantrine. The drug content of the selected formulations was found to be highest in S5 formulation (99.25%) and lowest in S1 (96.45%), the content in all the formulation suggested uniform distribution of drug. The drug content Artemether and Lumefantrine is mentioned in Table 3.

Table 3: The drug content Artemether and Lumefantrine

Sr. No.	Formulation code	Drug content Artemether	Drug content Lumefantrine
1	S1	95.33±0.30%	96.45±0.24%
2	S2	97.23±0.40%	96.32±0.65%
3	S3	98.23±0.40%	97.35±0.89%
4	S4	97.56±0.31%	95.34±0.24%
5	S5	98.71±0.56%	98.25±0.23%
6	S6	97.34±0.76%	97.81±0.12%

(Mean ± S.D, n = 3)

Degradation Study of Artemether and Lumefantrine due to Mixing or Wet-Milling

The thermal energy generated during wet-milling is lower than that generated by dry-mills because drugs are suspended in aqueous solution. It is generally considered that heat labile drugs are degraded by the heat generated during dry-milling. Intact Artemether and Lumefantrine in Nanopowder were quantified by HPLC analysis. The amount of intact Artemether and Lumefantrine in selected sample was found to be the same as in the control sample. This confirmed that suspension was not degraded by physical mixing and wet-milling process and is mentioned in Table 4.

Table 4 Degradation Study of Artemether and Lumefantrine

Sr.No.	Formulation code	Artemether Degraded in %	Lumefantrine Degraded in %	Limit of degradation
1	S5 post milled	2±0.25%	3±0.76%	5-20 %

(Mean ± S.D, n = 3)

The formulation with smallest particle size and high drug content was further subjected to further process of Lyophilization

Formulation of dry Nano powder

Normally, lyophilization is the recommended method to stabilize particles over months or years. However, resuspension of lyophilized Nanopowder is often difficult, especially when a stabilizer is not added. Nano suspension was converted to Nanopowder using freeze drying technique. Lyophilisation was done in order to avoid aggregation or caking of settled drug formulation. However, during the drying process, the particle aggregation should be considered since the benefits of nano-sized particles may be lost if the particle aggregation occurs. Therefore, an addition of protectants (usually sugars) may reduce the growth of particle size during a solidification process.

Evaluation of Nanopowder

Organoleptic properties of lyophilized Nano powder

Lyophilized Nanopowder (LF1) was visually inspected for color, odor and texture.

Appearance: Light Yellow powder

Odor : Slight

Particle size analysis

The nanoparticles in suspension tend to aggregate, thus forming undesirable larger-particle complexes. In this study, all Nanopowders formed nano-sized particles after resuspension, although the particle sizes increased slightly compared to those found in initial milled slurries. The lyophilized dried suspension exhibited good redispersibility upon ultrasonication. The particle size of lyophilized nanosuspension was found to be larger as compared to nanosuspension. The Particle size of lyophilized nanoparticles is mentioned in Figure 9. The particle size analysis of Formulation LS5 is found to be 732.9 nm

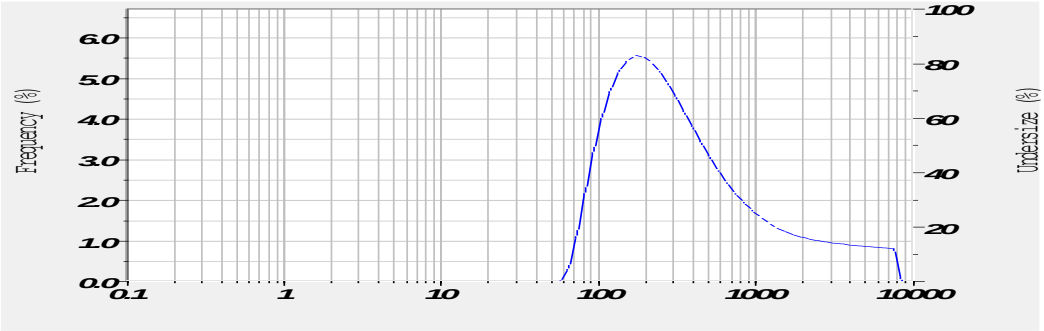

Figure 9: Particle size Analysis of Formulation LS5

Zeta potential

Zeta potential graph of formulation LS5 is mentioned in Figure 10.The negative values of zeta potential, which is reported to be the minimum to stabilize the colloidal particles by electrostatic repulsion, were given by using SLS as a surfactant. It is observed that stable and non-agglomeration of nanoparticles, the zeta potential value should be in the range of -30 to +30.

The zeta potential of lyophilized nanoparticles was found to be -0.2 mV indicating stability and nonagglomerating tendency of powder [51].

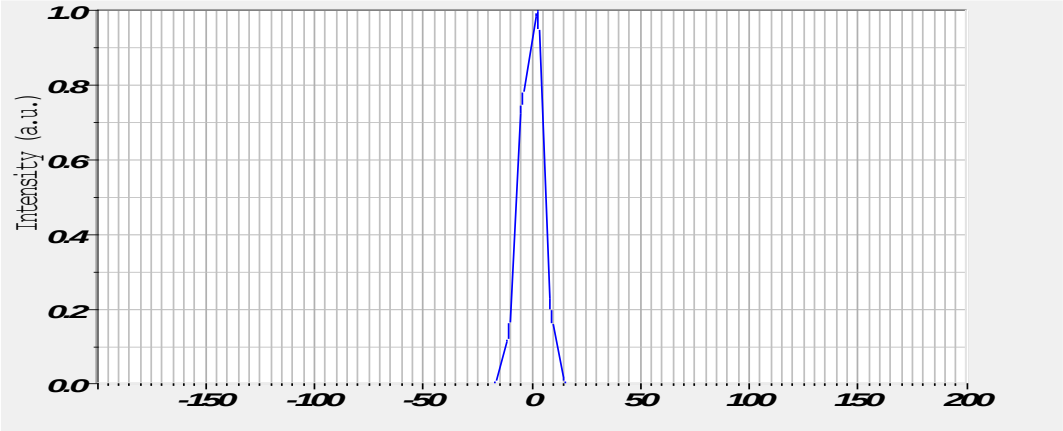

Figure 10: Zeta potential of Formulation LS5

Drug release [*in vitro*]

In vitro dissolution studies were performed for all samples for the determination of drug release profile due to poor aqueous solubility, oral bioavailability (40%), risk of degradation in acidic conditions; and associated risk of toxicity. Artemether was dissolved in buffer. Generally, in dissolution studies of hydrophobic drug, surfactant is added to maintain sink condition and to prevent precipitation of drug-in dissolution media.

The percent drug release of Artemether and Lumefantrine from different formulation batches, marketed formulation (Lumerax®) and pure drug is depicted in Figure 11 and Figure 12 respectively. The drug dissolution studies of Nano powder, marketed formulation (Lumerax®) and pure drug for Artemether and Lumefantrine is given in Table 5 and Table 6 respectively.

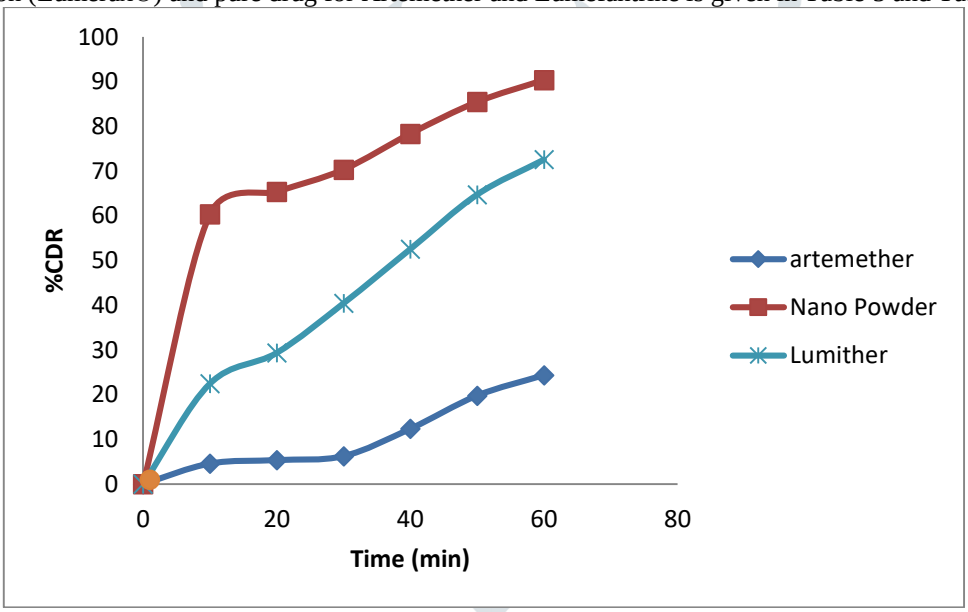

Figure 11 Cumulative drug release of Nano powder, marketed formulation and pure drug Artemether

Table 5 Cumulative drug release of Nano powder, marketed formulation (Lumerax®) and pure drug Artemether

Sr.No.	Time in minutes	% CDR Pure Artemether	% CDR Nano powder	% CDR Lumerax ®
1	0	0	0	0
2	10	4.56±0.39	59.21±0.70	22.45±0.08
3	20	5.34±0.98	65.34±0.29	29.34±0.40
4	30	6.23±0.77	70.31±0.09	40.43±0.45
5	40	12.35±0.76	78.34±0.59	52.56±0.87
6	50	19.76±0.23	85.43±0.65	64.74±0.62
7	60	24.34±0.96	90.34±0.21	72.56±0.12

(Mean ± S.D, n = 3)

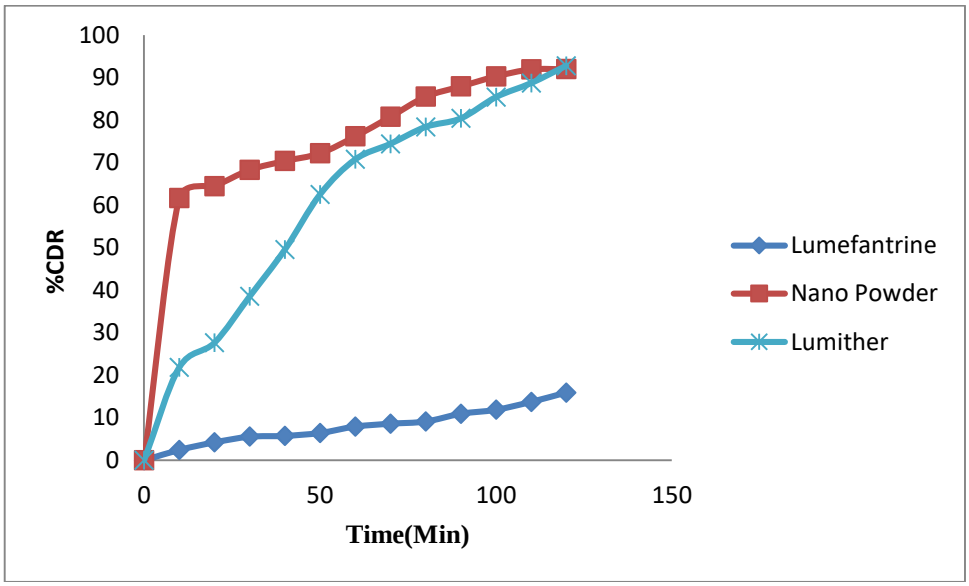

Figure 12: Cumulative drug release of Nano powder, marketed formulation and pure drug Lumefantrine

Table: 6 Cumulative drug release of Nano powder, marketed formulation and pure drug Lumefantrine

Sr. No.	Time in minutes	% CDR Pure Lumefantrine	% CDR Nano powder	% CDR Lumerax ®
1	0	0	0	0
2	10	2.46±0.56	61.67±0.81	21.87±0.71
3	20	4.23±0.76	64.52±0.60	27.67±0.44
4	30	5.56±0.05	68.34±0.45	38.56±0.54
5	40	5.76±0.09	70.43±0.02	49.56±0.09
6	50	6.44±0.21	72.24±0.21	62.54±0.76
7	60	7.94±0.12	76.21±0.29	70.78±0.83
8	70	8.62±0.08	80.83±0.62	74.45±0.86
9	80	9.13±0.72	85.54±0.01	78.43±0.49
10	90	10.94±0.87	87.98±0.23	80.43±0.05
11	100	11.89±0.21	90.34±0.89	85.44±0.09
12	110	13.74±0.98	91.98±0.21	88.74±0.12
13	120	15.94±0.02	92.03±0.72	92.83±0.09

(Mean ± S.D, n = 3)

SLN stabilized with surfactant mixtures were previously reported to have lower particle size and higher storage stability[51]and may be due to the formation of hybrid surfactant sheathing the surface spherical shaped[52].SLN were lyophilized to obtain dried systems, however, lyophilization can damage the surfactant film coating SLN surface due to freezing out effect, which may also cause particle aggregation during re-dispersion process [53] which may be the reason for less dissolution as compared to though the entrapment efficacy of Nanopowder was high.

Thus, from dissolution studies it can be concluded that aqueous solubility and dissolution rate of prepared formulation batches was significantly enhanced. Prepared nano powder was found to contain all the superior characters which were required and hence was further characterized.

Characterization of optimized formulation

Differential Scanning Calorimetry

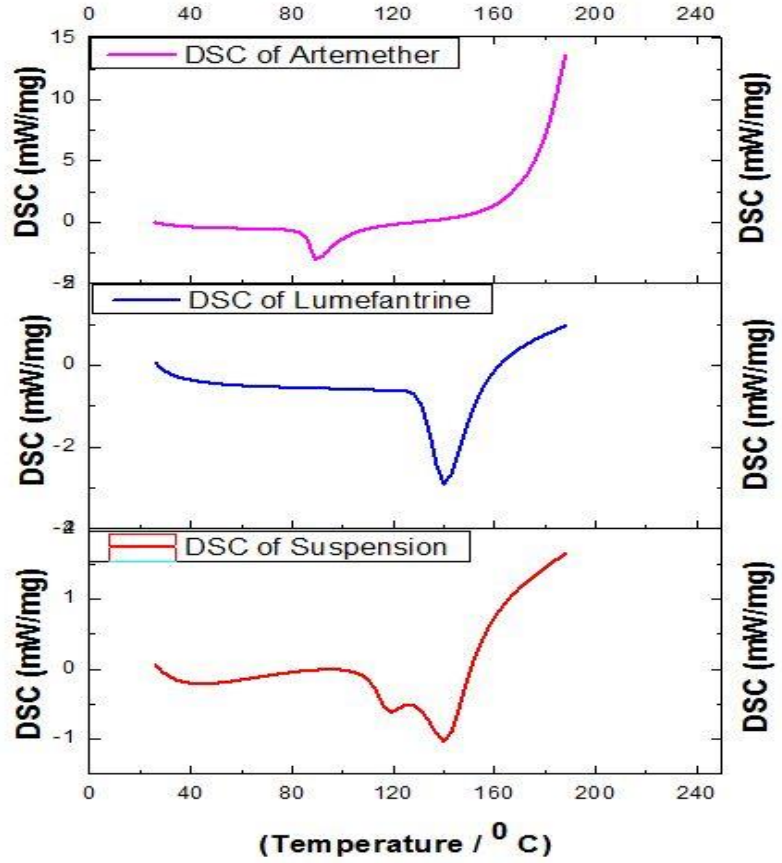

Figure 13: Differential Scanning Calorigraph of Artemether , Lumefantrine and Nanopowder

DSC measures the difference in the heat flow rate between the sample and the reference, when both are subjected to identical controlled temperature program. The DSC thermogram of Artemether showed typical characteristics of a crystalline substance indicated sharp endothermic peak at 89.7°C. Lumefantrine showed a sharp endothermal peak at 140.1°C and onset at 131.1 °C to its melting point which is identical to its literature value. Differential scanning calorimetry of pure drugs Artemether and Lumefantrine represented sharp endotherm peak at their melting points and Nanopowder represented no such peak which indicated change in melting behaviour of drug and retention of crystallization. The disappearance of the melting endotherm in the DSC scan of Nanopowder of Artemether and Lumefantrine is attributed to reduction of particle size with enhanced surface area leading to change in enthalpy of formulation due to presence of excipients. Differential scanning calorigraph ofThe Differential Scanning Calorigraph of Artemether, Lumefantrine and nanopowder is given in Figure 13.

Scanning Electron Microscopy

The samples were observed for morphological characterization. Scanning Electron Microscopy was carried out for comparison of surface of pure drug Artemether and Lumefantrine with the Nanopowder. The pure Artemether was characterized by crystals of bigger size and regular shape with an apparently smooth surface. SEM micrographs of Lumefantrine revealed large crystalline blocks characterizing its identity and crystalline character. The SEM micrographs of Artemether and Lumefantrine are mentioned in Figure 14 and Figure 15 respectively.

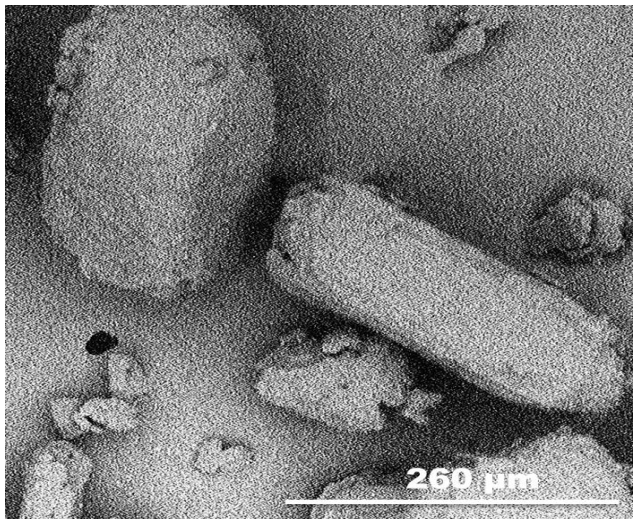

Figure 14: Scanning Electron Micrograph of Artemether

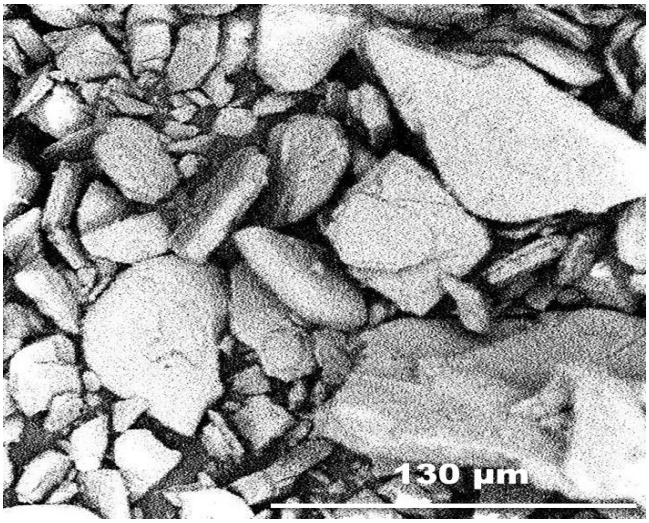

Figure 15: Scanning Electron Micrograph of Lumefantrine

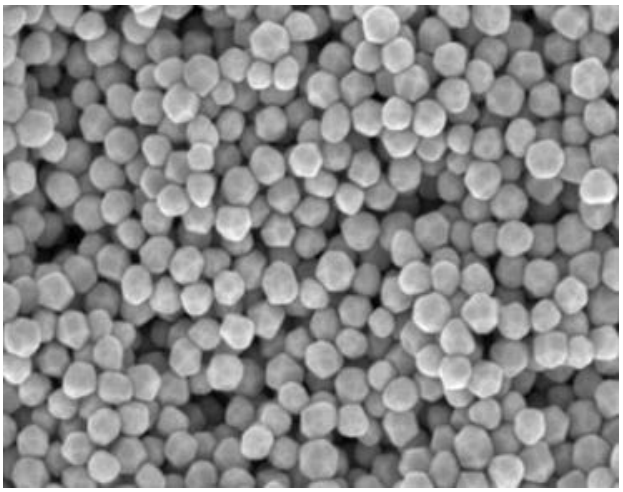

Figure 16: Scanning Electron Micrograph of Nanopowders

Pure drugs appeared under the scanning electron microscope having rough surfaces and crystalline forms. However the SEM of its Nanopowder indicated that all the particles were found to be roughly spherical in shape with a well-defined periphery. The Nanopowder indicated no agglomeration in SEM image due to Stabilizer in coating surface and further giving small particle size. The surface morphology of Nanopowder indicated that intact nature of Nanopowder would aid enhanced solubilisation. Scanning Electron Micrograph of Nanopowder is presented in Figure 16.

Infrared spectroscopy

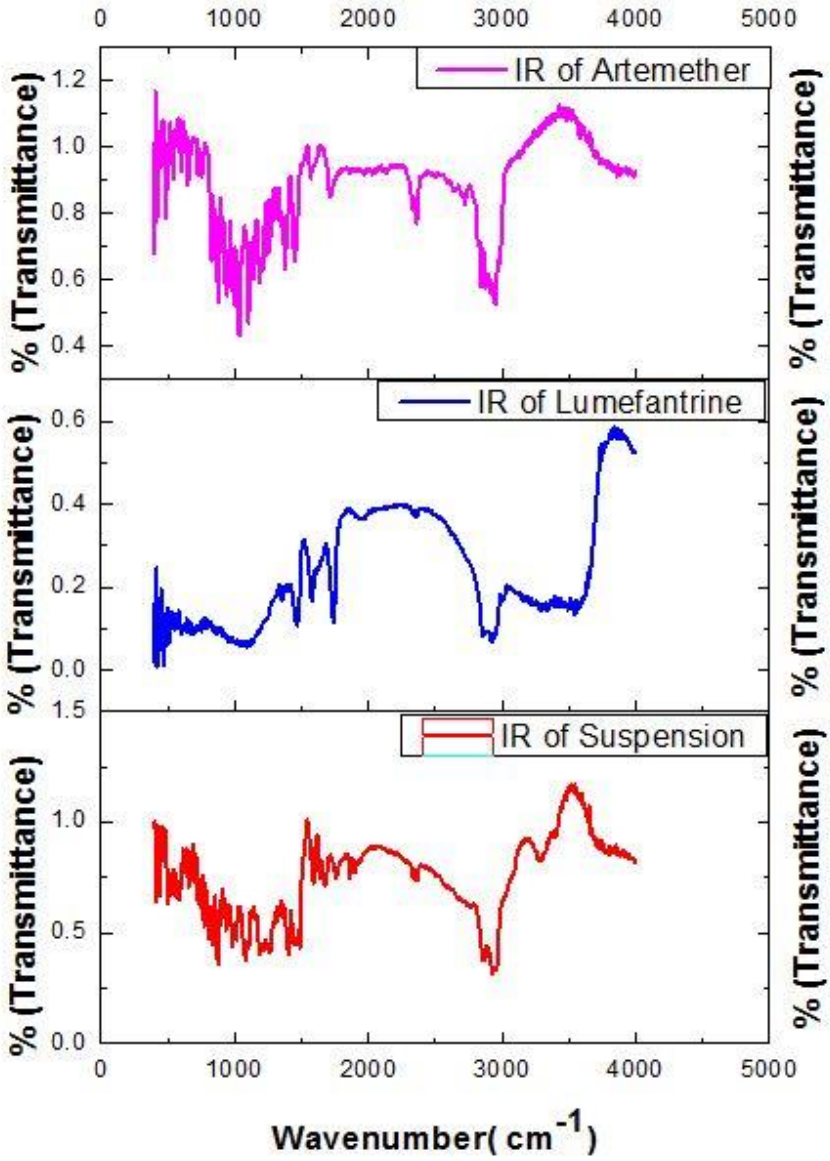

Figure 17: Infrared Spectrum of Artemether , Lumefantrine and Nano Powder

Table 7: Interpretation of IR Spectrum of Artemether

Sr. No.	Wavenumber (cm^{-1})	Group	Stretching/ Deformation
1	2949.30	$-CH_2$, $-CH_3$	Stretching aliphatic
2	1157.3	C-O-C	Stretching (ether linkage)
3	1375.38-1450.77	$-CH_2$, $-CH_3$	Bending vibrations
4	651.22	=C–H	Bending

Table 8: Interpretation of IR Spectrum of Lumefantrine

Sr. No.	Wavenumber (cm^{-1})	Group	Stretching/ Deformation
1	3402.70	O-H	Aromatic Stretching
2	1155.86	C-O	Stretching
3	2955.75	C-H	Aliphatic Stretching
4	3094	C-H	Aromatic Stretching

IR spectrum of Lumefantrine revealed the presence of major functional groups present in the structure of Lumefantrine supporting its identity

FT-IR was used to study the drug excipient compatibility. FT-IR spectra of nanopowder revealed no considerable change in major peaks when compared to FT-IR of pure drug which proved that there was no interaction between drug and excipients. Overall there was no chemical interference of functional groups between and there was no change in functional properties of drugs**.** Interpretation of FT-IR spectrum of of Artemether, Lumefantrine, Nanopowder of Artemether and Lumefantrine is mentioned in Table 7-9 respectively. Infrared spectrum of Artemether, Lumefantrine and Nanopowderis shown in Figure 17.

Table 9: Interpretation of IR Spectrum of Nanopowder

Sr. No.	Wavenumber (cm^{-1})	Group	Stretching/ Deformation
1	2948.98	-CH2,-CH3	Aliphatic Stretching of Artemether
2	1112.46	C-O-C	Ether stretching in Artemether
3	1315.64-1438.72	-CH2,-CH3	Bending vibrations of Artemether
4	932.80	=CH-H	Alkene Bending
5	2948.98	C-H	Aromatic Stretching of Lumefantrine
6	1041.84	C-O	Stretching of Lumefantrine
7	2905.36	C-H	Aliphatic Stretching in Lumefantrine
8	648.72-788.15	=C-H	Bending Alkene in Lumefantrine
9	1737.23	C=O	Bending Ester in Lumefantrine

X- ray Diffraction

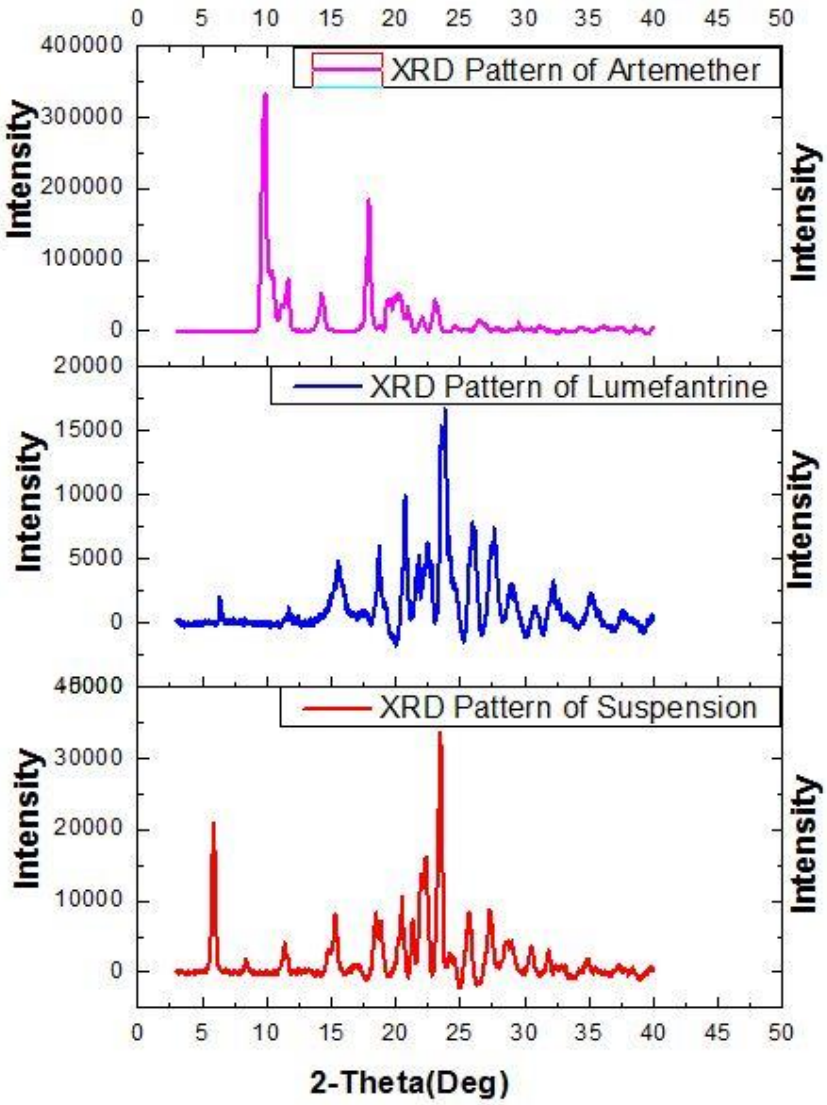

Figure 18: X-ray diffraction pattern of Artemether, Lumefantrine and Nanopowder of Artemether and Lumefantrine

The X-ray diffractogram of Artemether verified the physical nature of Artemether; the drug represented numerous intense and sharp multiple peaks corresponding to crystalline nature of drug. The XRD patterns of Artemether showed very strong characteristic diffraction peaks at 2θ of 9.88°, 17.64°, 18.04° and 19.68°. It signifies that Artemether is purely a crystalline compound.

The XRD of Lumefantrine indicated specific peaks of crystallinity at 2θ of 6.9°, 8.5°, 10.5°, 12.91°, 13.64°, 18.12°, 19.21°, 20.72°, 21.6°and 32.14° indicating its crystalline structure.

X-ray diffraction pattern of Nanopowder of Artemether and Lumefantrine verified the crystal transformation pattern of the drug in the Nanopowder. Pure drugs represented sharp peak which indicated it was highly crystalline in nature and formulation depicted significant crystalline peaks of drugs indicating that the physical state of drugs remained unchanged.

X-ray crystallography of Artemether , Lumefantrine and Nanopowder is depicted in Figure 18.

IV. CONCLUSION

In this research solid lipid nanoparticles were successfully prepared and using high pressure homogenization in laboratory of Artemether and Lumefantrine. The developed techniques were simple, reproducible, prepared nanoparticles without the need of organic solvents or any sophisticated instruments and have the potential to easily scale up for large scale production.

The formulation with smallest particle size and highest entrapment efficacy was further carried for lyophilisation. The Nanopowder dispersions were lyophilized to stabilize the solid lipid nanoparticles and the lyophilized exhibited good redispersibility upon ultrasonication.

Thus, the problem of efficiently delivering poorly water soluble drugs could be solved by such innovative lipid based drug delivery system that may increase their solubility and bioavailability.

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