SOLUBILITY AND BIO-AVAILABILITY ENHANCEMENT OF ANTIMALARIAL DRUGS: ARTEMETHER AND LUMEFANTRINE THROUGH SOLID LIPID NANO PARTICLES

Sanjana Gaikwad[1]*, Madhukar Tajne[1]

[1*]PhD Research Scholar, University Department of Pharmaceutical Science, Rashtrasant Tukadoji Maharaj Nagpur University, University Campus, Amravati Road, Nagpur -440033, Maharashtra, India.

[1]Professor, University Department of Pharmaceutical Science, Rashtrasant Tukadoji Maharaj Nagpur University, University Campus, Amravati Road, Nagpur -440033, Maharashtra, India.

Abstract: Present study describes the formulation of Solid lipid nanoparticles (SLNs) System of Antimalarial Drugs Artemether and Lumefantrine with lipids and surfactants which enhances the solubility and bioavailability. They consist of spherical lipid particles in nanometer size range. Artemether Lumefantrine loaded lipid nano particles composed of lipid mass produced by high pressure homogenization method using Lipid phase : Glyceryl trimyristate, Soyabean Oil, Surfactant phase: Tween 80. SLNs were further characterized for particle size, Zeta Potential; Percent encapsulation efficiency reported optimized values to be 157.6 nm, -0.2 mV, 98. 45± 0.11 of Artemether and 93.36 ± 0.10 of Lumefantrine. In-vitro Diffusion Studies reported to be 95.9 % for Artemether and 93.86% for Lumefantrine. The in vitro percent drug release of Artemether and Lumefantrine from SLN'S 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.

Keywords: **Antimalarials, Nanotechnology SLNs, Enhanced solubility, Dissolution, Zeta Potential, Lumefantrine.**

I. INTRODUCTION

Colloidal particles extending in measure in the vicinity of 10 and 1000 nm are known as nanoparticles. They are made from natural or synthetic polymers and preferably suited to improve conveyance and lower toxicity. Throughout the years, they have risen as a variable substitute to liposomes as medication transporters. The effective execution of nanoparticles for drug conveyance relies upon their capacity to enter through a few anatomical boundaries, supported arrival of their substance and their soundness in the nanometer measure. To beat these confinements of polymeric nanoparticles, lipids have been advanced as an option transporter, especially for lipophilic pharmaceuticals. These lipid nanoparticles are known as strong lipid nanoparticles (SLNs), which are pulling in wide consideration of formulators around the world.

SLNs are colloidal transporters created in the most recent decade as an option framework to the current customary bearers (emulsions, liposomes and polymeric nanoparticles). They are another age of submicron-sized lipid emulsions where the fluid lipid (oil) has been substituted by a strong lipid. SLN offer exceptional properties, for example, little size, extensive surface territory, high medication stacking and the cooperation of stages at the interfaces, and are attractive for their capability to enhance execution of pharmaceuticals, neutraceuticals and different materials

SLNs possess a solid lipid core matrix that can solubilize lipophilic molecules. The SLNs are sub-micron colloidal bearer which is made out of physiological lipid, scattered in water or in an aqueous surfactant. They are comprised of strong

hydrophobic center having a monolayer of phospholipids covering. Strong center contains the medication scattered or disintegrated in lipid network. They can possibly convey lipophilic or hydrophilic medications. The lipid core is stabilized by surfactants (emulsifiers).Structure of SLNs is depicted in **Figure 1**. The term lipid is used here in a broader sense and includes triglycerides, diglycerides, monoglycerides, fatty acids, steroids, and waxes. All classes of emulsifiers (with respect to charge and molecular weight) have been used to stabilize the lipid dispersion. It has been found that the combination of emulsifiers might prevent particle agglomeration more efficiently [1].

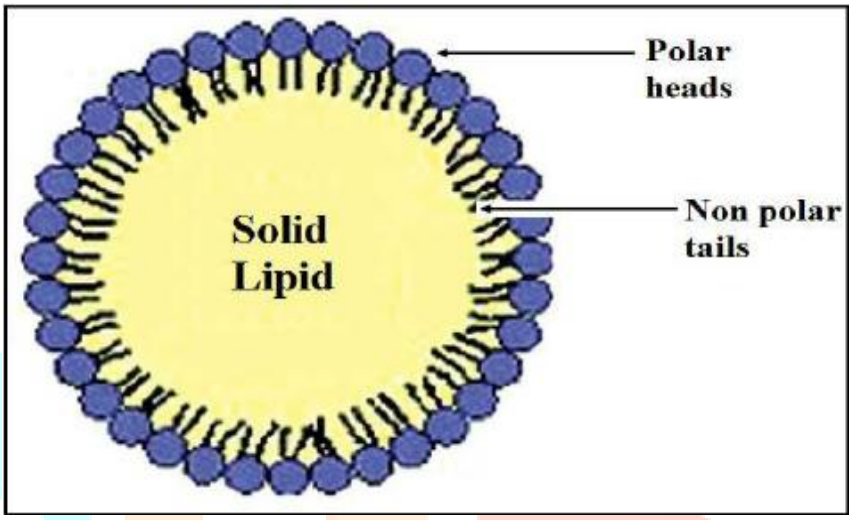

Figure: 1 Proposed structure of SLNs

Each of the right now researched particulate bearers (polymeric nanoparticles, fat emulsion, liposomes) has particular favorable advantages and disadvantages. The particulate colloidal bearers experience the ill effects of specific disservices like moderate degradation which can cause harmful impacts on reticuloendothelial cells. Creation of colloidal bearers by dissolvable dissipation produces dangerous deposits. The biodegradable polymers Polylactide and Polylactide/glycolide utilized as a part of generation of colloidal bearers could cause cytotoxic impacts after phagocytosis. Gamma illumination utilized for disinfection can cause the arrangement of unsatisfactory harmful response items; also the scale-up techniques are not accessible. Interestingly the SLNs have the edge of having certain particular points of interest like low costs, simplicity of planning and scale up. They have high dispersibility in a fluid medium and high entrapment of hydrophobic medications with controlled molecule estimate and broadened arrival of entangled medication. Physiological lipids are utilized in assembling of strong lipid nanoparticles which offers high biocompatibility and biodegradability to the framework with great stability. They have additionally demonstrated particular and wide potential application range which incorporates dermal and intravenous organization [2].

1.1 Advantages of SLN

The advantages of SLNs are as follows:

1. Their small size and relatively narrow size distribution permits site specific drug delivery.
2. Controlled and sustained release of active drug can be achieved.
3. The incorporated drug is protected from the onslaughts of biochemical degradation.
4. It can be lyophilized.
5. It is relatively cheap and stable.
6. It can be used as physiological lipids,
7. There is avoidance of organic solvents [3].

Solid lipid nanoparticles (SLNs) have been utilized as an option drug delivery framework to colloidal drug delivery systems specifically oil-in-water emulsions, liposomes, micro particles and polymeric nanoparticles. They comprise of lipid particles in nanometer range. SLNs are comprised of solid lipids, emulsifier or potentially co-emulsifier and water. An ideal solid lipid liquefies at temperatures surpassing body temperature (37°C).

Antimalarials combination therapy which has been broadly investigated by the exploration researchers includes concurrent utilization of at least two blood schizontocidal drugs with free methods of activity against biochemical focuses in the parasite. Among the created mixes, WHO suggest couple of levelheaded mixes and Artemether – Lumefantrine is one of them. The method of reasoning for consolidating these two Antimalarials with various methods of activity was to couple the synergistic quick beginning of activity of Artemether with the long term of activity of Lumefantrine. Artemether is basic for quick clearance of parasitaemia and fast determination of side effects. It lessens parasite numbers by a factor of roughly 10,000 in each a biogenetic cycle, which is more than other current Antimalarials (which decrease parasite numbers 100– 1000 overlay for each cycle). Artemether is successful against sedate safe jungle fever and also it decreases gametocyte carriage. However the medication shows a short half-existence of 2– 3 h. This downside is dealt with by joining it with Lumefantrine which acts gradually and has a more drawn out half-life. This long-acting impact of Lumefantrine is thought to counteract recrudescence .Artemether and Lumefantrine together help to decrease the specific weight on the parasite to create protection.

Malaria is an intense febrile disease. In a non-safe individual, side effects seem seven days or all the more (for the most part in the vicinity of 10 and 15 days) after the infective mosquito bite. The principal side effects fever, cerebral pain, chills and retching might be gentle and hard to perceive as jungle fever. If not treated inside 24 hours, P. falciparum intestinal sickness can advance to extreme disease, frequently prompting demise [4].

In a meal full of fat, Lumefantrine demonstrates unpredictable assimilation and fundamentally expanded bioavailability (roughly 16-overlay). In the event of Artemether, bioavailability increments by 2-fold.Artemether is a Biopharmaceutics Classification System (BCS) Class II tranquilize displaying low fluid dissolvability with higher penetrability and furthermore gets immediately used in GIT, while Lumefantrine has low solvency and low porousness (BCS Class IV).Thus, primary challenge is to design an oral formulation which not only enhances the solubility of both the drugs but also overcomes the metabolism of Artemether in the GIT with enhanced permeability of Lumefantrine . To overcome these biopharmaceutical challenges, adaptable definition approaches which will hold the physicochemical properties of the individual medications while at the same time beating the physiological difficulties are required.

Lipid based medication conveyance frameworks have been exhibited to be valuable in upgrading the bioavailability of such BCS Class II particles. Since these lipids based excipients keep the medication in the disintegrated state until the point when it is consumed, they defeat the hindrance of moderate disintegration rates. Lipids are a standout amongst the most adaptable excipient classes right now accessible, furnishing the formulator with numerous potential choices for enhancing and controlling the assimilation of inadequately water-solvent medications. In this manner, the present work is centered around improvement of lipid based medication conveyance frameworks of Artemether and Lumefantrine in mix to build the dissolvability of both the medications, which could likely encourage the assimilation of medications and beat the present disadvantage of conflicting bioavailability [5].

II. MATERIALS AND METHODS

Artemether was provided as a gift sample by Ipca Laboratories Ltd., Mumbai, India. Lumefantrine was provided as a gift sample by Zim Laboratories, Nagpur, India. Cremophor EL was obtained as a gift sample from BASF, Mumbai, India. Tween 80 and Oleic acid was purchased from Merck India Ltd, Mumbai, India.

2.1 METHODOLOGY

2.1.1 Preparation of SLN'S

The formulation and development of SLN consisted of screening of lipids, selection of formulation ingredients and preparation of SLN.

2.1.2 Screening of Lipid

Liquid screening test was performed, prior to the formulation of SLN, to determine the most suitable lipid for the active ingredient to be incorporated in SLN. The solubility of Artemether and Lumefantrine was determined in different solid lipid lipids. The solubility of drug in melted solid lipid is one of the most important factors that determine the loading capacity of drug in lipid. The solubility studies were carried in Soyabean oil, Stearic acid, Glyceryl monostearate, Glyceryl trimyristate, Transcutol P and Tween 80.

The weighed amount of solid lipid was added into the test tubes which were heated in a controlled temperature kept at 10^0C above solid lipid melting point, with gentle shaking, 100 mg of each Artemether and Lumefantrine were added separately in small quantity till turbidity or crystals of drug were seen. The remaining amount of Artemether and Lumefantrine was weighed again and solubility of drug was determined in mg/g of solid lipid and liquid lipid using HPLC. Lipid showing maximum solubility of Artemether and Lumefantrine was selected for preparation of Solid lipid nanoparticles [6].

2.1.3 Formulation ingredients

Selection of the components for solid lipid carrier system was based on solubilizing capacity of the excipient. The selected components were as follows:

Lipid phase : Glyceryl trimyristate, Soyabean Oil

Surfactant phase: Tween 80

Aqueous phase : Double Distilled Water

Formulation ingredients used in preparation of SLN were used in concentrations is shown in **Table 1.**

TABLE 1: FORMULATION OF SOLID LIPID NANOPARTICLES

SR. NO.	INGREDIENTS	FORMULATION CODE				
		PF1	PF2	PF3	PF4	PF5
1	Glyceryl trimyristate	1%	1.5%	2%	2.5%	3%
2	Soyabean oil	1%	1.%	1%	1%	1%
3	Tween 80	1%	1%	1%	1%	1%
4	Distilled Water	qs	qs	qs	qs	qs

2.1.4 Method of preparation of SLN

Solid lipid nanoparticles (SLN) can be prepared by various methods. But the method of choice adopted for the formulation of drug loaded nanoparticles was high pressure homogenization since it is commercially for several years. Preparation of SLN containing Artemether and Lumefantrine was processed by hot High Pressure Homogenization (HPH) process, as depicted in **Figure 2.**

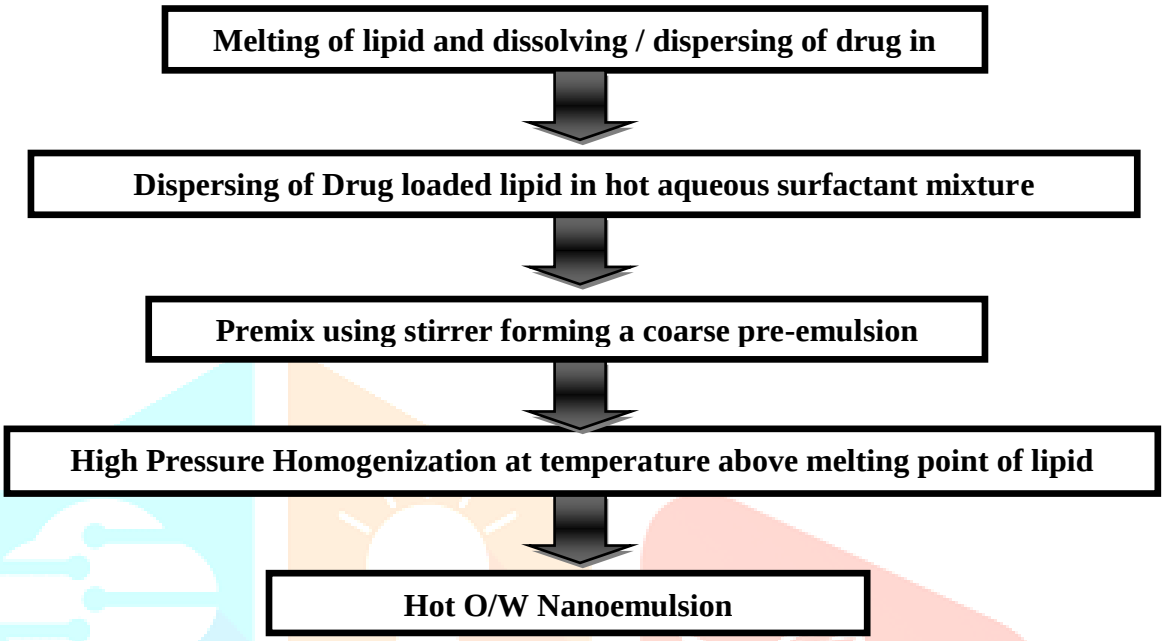

FIGURE 2: FLOW CHAT FOR THE PREPARATION OF NANOPARTICLES BY HPH

Briefly, Glyceryl trimyristate which comprised of the lipid phase was kept in a heating water bath. Then to the molten lipid phase, weighed amount of Artemether and Lumefantrine were added. The aqueous phase comprised of surfactant dissolved in distilled water. Both the phases were maintained at a temperature 10^0C above the melting point of the lipid.

At this temperature, the melted hot lipid phase was then dispersed in hot surfactant phase, obtaining a pre-emulsion under mechanical stirring at 900 rpm for 30 minutes. Then this warm pre-emulsion was introduced into high pressure homogenizer at 800 bar pressure and 6 cycles to form the SLN dispersion. Then SLN dispersion so formed was allowed to cool at room temperature which was further used for characterization.

The lipid nanoparticles stabilized with surfactant Tween 80 which has lower particle sizes and higher storage stability. For this reason Tween 80 was used as surfactant. On the basis of results it was suggested that 1% w/v Tween 80 was sufficient to cover the surface of nanoparticles effectively and prevent agglomeration during the homogenization process [7].

III. CHARACTERIZATION OF NANOPARTICLES

The prepared SLN dispersions were evaluated for particle size and polydispersibility index, zeta potential, %DL and %EE. Depending on these results optimized batch selection was done.

3.1 Particle size and poly dispersibility index

The droplet size and polydispersibility index of the emulsions was determined by Particle size analyzer. Emulsions (0.2 ml) were diluted to 100 ml with distilled water.

3.2 Zeta Potential

The Zeta Potential (ZP) reflects the electric charge on the particle surface indicating the physical stability of colloidal systems. The zeta potential measurement was performed using a Particle size analyzer. Adjusting conductivity of distilled water used for diluting samples avoids the fluctuations of the zeta potential due to variations in conductivity.

3.3 Percent encapsulation efficiency and loading capacity

Percent Encapsulation efficiency is defined as the percentage of drug incorporated into the lipid nanoparticles relative to the total drug added. It specifies how much percent of drug are included in the particles and how much percent of free drug is still present in the dispersion medium.

Loading capacity refers to the percentage of drug incorporated into the lipid nanoparticles relative to the total weight of the lipodial phase (i.e. lipid + drug). Percent encapsulation efficiency (%EE) was determined by measuring the concentration of the unentrapped free drug in dispersion. The aqueous medium was separated by centrifuged.

About 2ml of the dispersion was placed in the allomer tubes and centrifuged at 75,000 rpm for 45 minutes at 4^0C. The average entrapment efficiency and drug loading of the nanoparticles and standard deviation was calculated for each batch of nanoparticles (n=3). The percent encapsulation efficiency (%EE) and percent drug-loading (%DL) were calculated by using following formula 1 and 2 [8, 9].

$$\%\mathbf{EE} = \frac{\textbf{Amount of drug added} - \textbf{Amount of drug in supernatent}}{\textbf{Amount of drug added}} \textbf{x100}$$

$$\%\mathbf{DL} = \frac{\textbf{Amount of drug added} - \textbf{Amount of drug in supernatent}}{\textbf{Amount of lipid added}} \textbf{x 100}$$

3.4 Freeze-drying nanoparticles

SLN dispersions were freeze-dried to obtain dry product used for the analytical determination by thermal analysis. The SLN dispersions were fast frozen under -75^0C in the presence of Mannitol as cryoprotectant in varying concentrations in a deep-freezer for 1 h and then the sample were moved for the drying process in the freeze-drier. The drying period was 72 h by applying vacuum at 100 mTorr and then the SLN powders were reconstituted in ultra-purified water under gentle agitation and the mean particle size was evaluated.

IV. EVALUATION OF LYOPHILIZED SLN'S

4.1 Particle size analysis

The droplet size of Lyophilized SLN was determined by Particle size analyzer. SLN were diluted to 100 ml with distilled water sonicated for 15 min using ultrasonicator.

4.2 Zeta Potential

The Zeta Potential (ZP) reflects the electric charge on the particle surface indicating the physical stability of colloidal systems. The zeta potential measurements were performed using a Particle size analyzer.

Drug release study (in vitro)

Apparatus type:	USP XXII type II (paddle)
Dissolution medium:	900 ml in phosphate buffer [pH 7.2, with 1 %w/v SLS] and 0.1N HCl [pH-1.2]
Temperature of dissolution medium:	37±0.5°C
Speed of rotation of paddle:	50 rpm
Volume of sample withdrawn:	10 ml
Sampling interval:	Every 60 min

The in vitro dissolution studies were performed in order to ensure the quick release of the drug in the dissolution medium and they also act as an important quality control tool for the dosage forms. Furthermore, in vitro dissolution studies, required

quantity of solid lipid nano particles (equivalent to 120 mg of Artemether and 20 mg of Lumefantrine) and pure drug 120 mg of Artemether and 20 mg of Lumefantrine were used. Aliquots were withdrawn and analysed by HPLC for cumulative percentage drug release. Marketed formulation Lumirax® was also studied [10-11].

V. CHARACTERIZATION OF OPTIMIZED FORMULATION

5.1 Differential Scanning Calorimetry

The physical state of drugs and formulation was characterized by Differential Scanning Calorimetry. The sample was placed in standard Aluminum 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 behavior of drug.

5.2 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.

5.3 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 Aluminum stub. The stubs were then coated with Platinum to a thickness of above 10 Å 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.

5.4 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 kα radiation angles from 10°- 70° [12].

VI.RESULTS AND DISCUSSION

6.1 Formulation of SLN'S

Solid Lipid nanoparticles were prepared by High Pressure Homogenization technique. High Pressure Homogenization of the pre-emulsion was carried out at temperatures above the melting point of the lipid. In general, higher temperature results in lower particle sizes due to the decreased viscosity of inner phase. However, high temperatures increase the degradation rate of the drug and the carrier. Increasing the homogenization pressure or the number of cycles often results in an increase of the particle size due to high kinetic energy of the particles.

6.2 Screening of Lipid

Solubility of Artemether and Lumefantrine in solid lipids used is mentioned in **Table 2.**

TABLE 2: SOLUBILITY OF ARTEMETHER AND LUMEFANTRINE IN VARIOUS SOLID LIPIDS

SR. NO.	SOLID LIPID	SOLUBILITY OF ARTEMETHER (MG/G)	SOLUBILITY OF LUMEFANTRINE (MG/G)
1	Stearic acid	30.4± 0.21	20.6 ± 0.46

2	Glyceryl trimyristate	100.6± 0.32	60.2± 0.23
3	Glyceryl monostearate	24.45± 0.67	45.2 ± 0.14
4	Soyabean oil	75.87± 0.32	61.3 ± 0.59
5	Transcutol P	60.89± 0.54	27.3 ± 0.67

(Mean ± S.D, n = 3)

Depending on solubility Glyceryl trimyristate showed highest solubility of Artemether and Lumefantrine, making it most suitable for incorporation into solid lipid nanoparticles. The solubility was found to be in order Glyceryl trimyristate>Soyabean oil>Transcutol P> Stearic acid> Glyceryl monostearate for Artemether whereas solubility of Lumefantrine was found to be in order Soyabean oil>Glyceryl trimyristate>Glyceryl monostearate>Transcutol P>Stearic acid.

6.3 Preparation of SLN'S

The use of 1% of a single emulsifier has been shown to give coarse emulsions with high coalescence rate of the solvent droplets. In some rare cases a single emulsifier can yield the desired emulsion. More often, in the case of oil-in-water emulsions, mixed surfactants have been reported to have a synergistic effect on emulsion stability in term of coalescence rate. The combined use of two or more emulsifying agents appear to produce mixed surfactant films at the interface having high surfactant coverage as well as sufficient viscosity to promote stability[13].From screening of different solid lipids and lipid lipids the components were selected.

VII. EVALUATION OF SLN'S

7.1 Particle size and polydispersibility index

The physical stability of SLNs depends on their particle size. When the suspended particles are small, they diffuse relatively fast, and the fluctuations in the scattered light are rapid. On the other hand, if the particles are large, they move slowly, and the fluctuations in the scattered light are slow. The results obtained on particle size and polydispersity index are given in **Table 3.**

TABLE 3: PARTICLE SIZE AND POLYDISPERSITY INDEX OF FORMULATED SLN

SR.NO.	FORMULATION CODE	PARTICLE SIZE (NM)	POLYDISPERSITY INDEX
1	PF1	121.8	0.415
2	PF2	188.6	0.470
3	PF3	473	0.542
4	PF4	614.5	0.577
5	PF5	205.8	0.543

From **Table 3** it was observed that minimum particle size was observed in formulation batch **PF1** and maximum particle size was observed in formulation batch **PF4.** The particle size of formulation batches PF1, PF2, PF3, PF4 and PF5 are shown in Figure 3, 4, 5, 6 and 7 respectively.

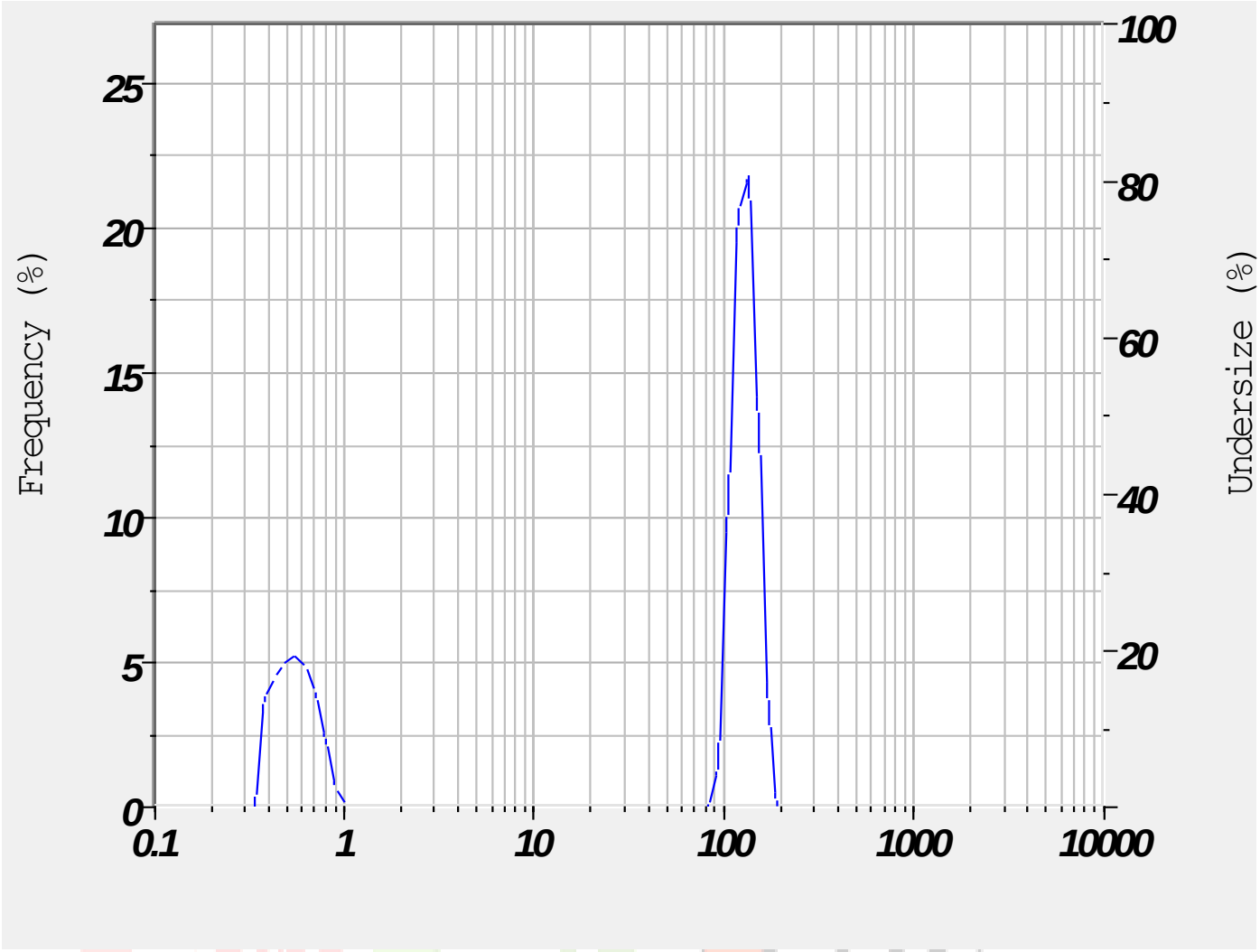

Figure 3: Particle size of formulation PF1

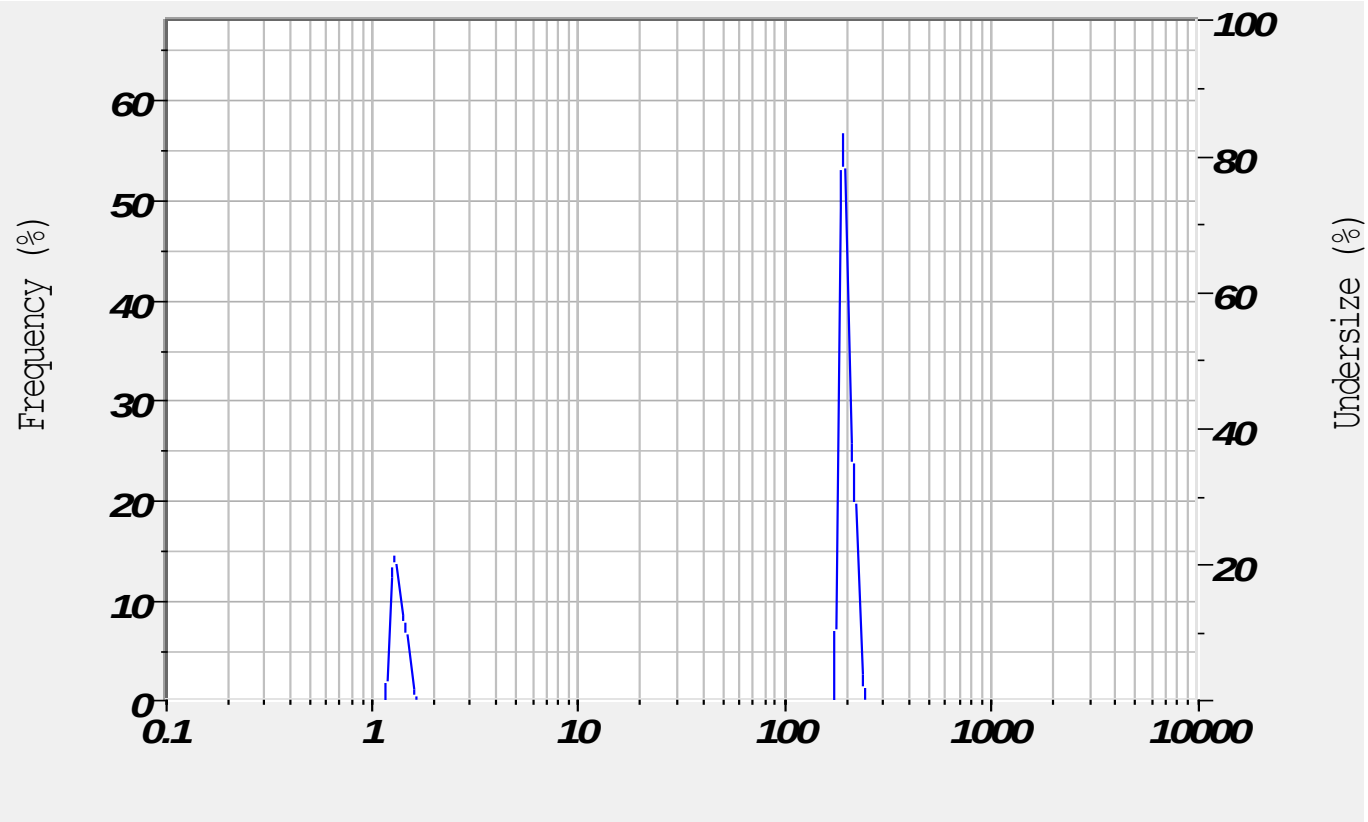

Figure 4: Particle size of formulation PF2

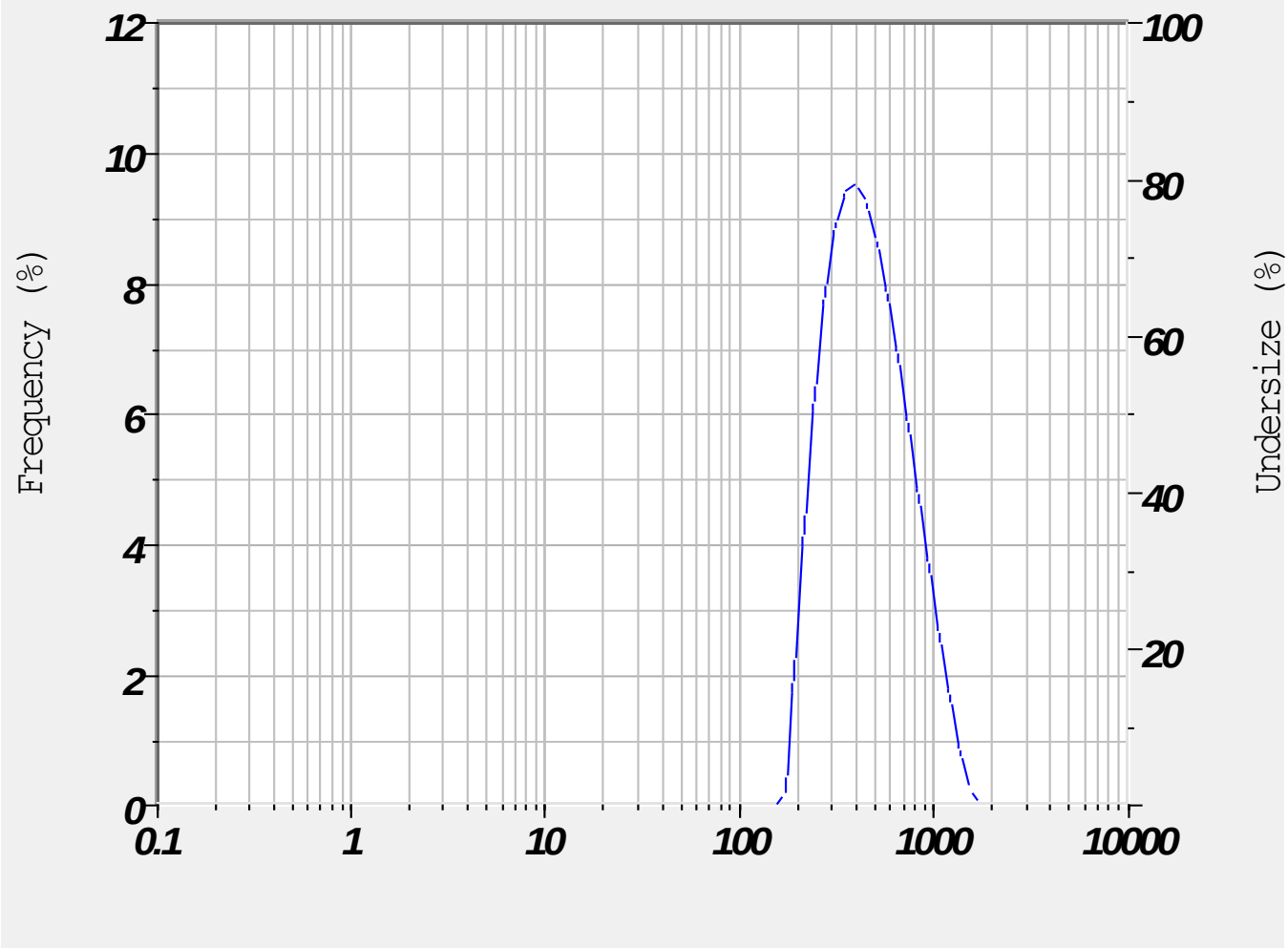

Figure 5: Particle size of formulation PF3

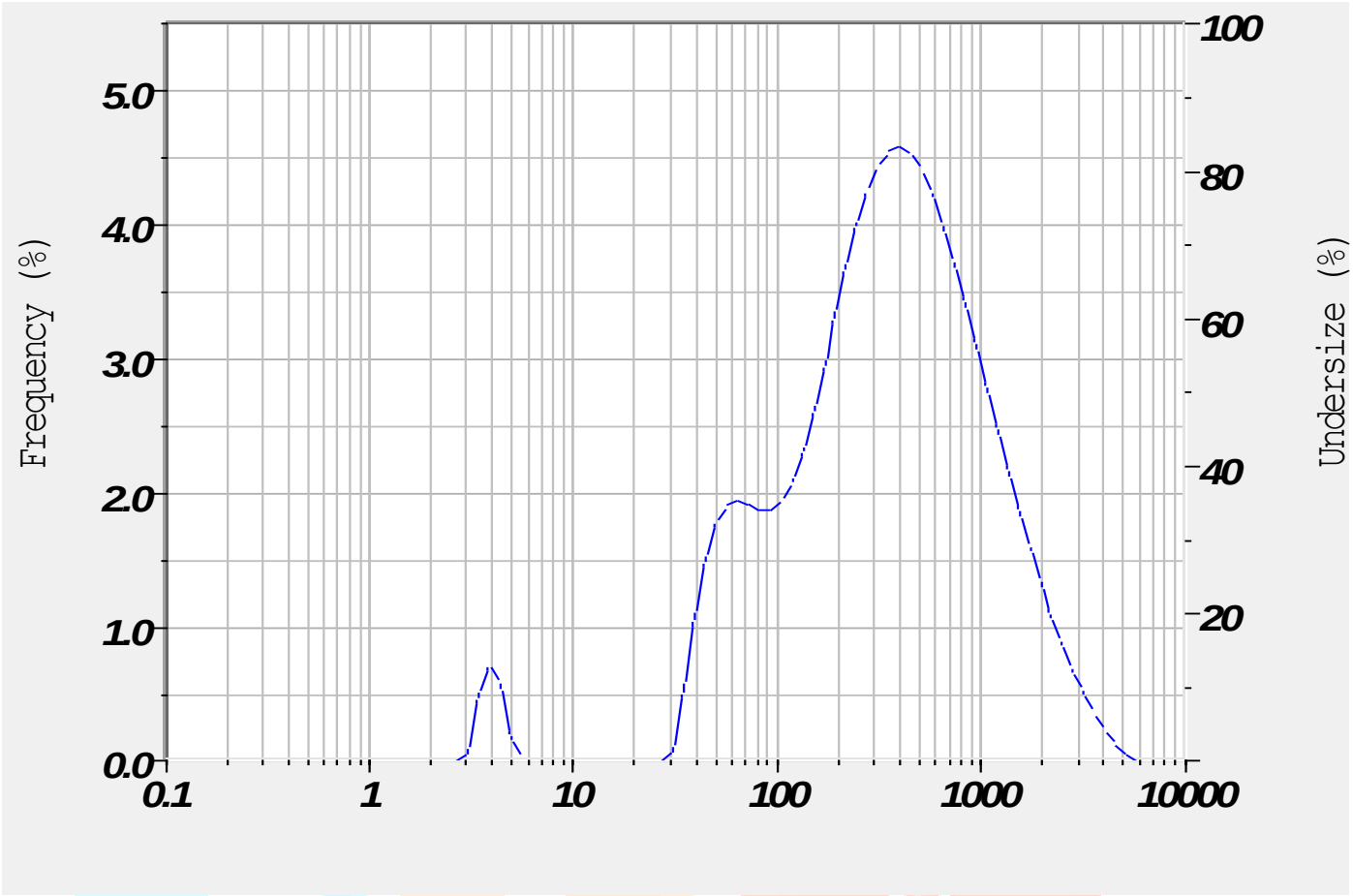

Figure 6: Particle size of formulation PF4

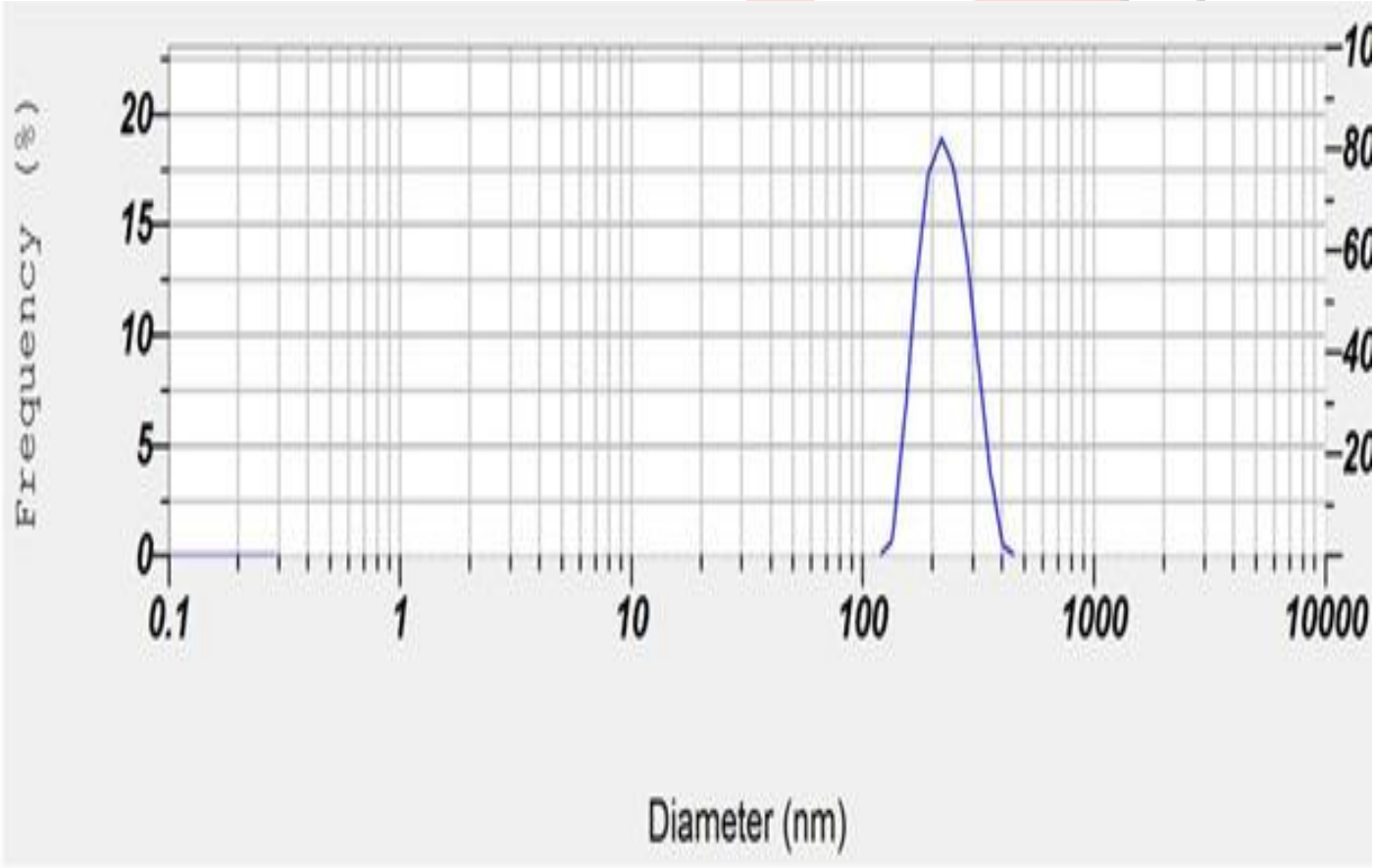

Figure 7: Particle size of formulation PF5

It was observed that increase in lipid concentration leads to a concentration dependant increase in particle size. Rising concentration of lipid increases viscosity of solvent phase which in turn may reduce diffusion rate of lipid molecules in the outer phase [14]. By observing **Table 1** and particle size distribution it can be concluded that increasing concentration of Glyceryl trimyristate from 1 to 3 % increases the particle size.

7.2 Zeta Potential

Zeta potential is an important product characteristic of SLNs since its high value is expected to lead to deaggregation of particles in the absence of other complicating factors such as steric stabilizers or hydrophilic surface appendages. Zeta potential measurements allow predictions about the storage stability of colloidal dispersions. The Zeta Potential was found to be in range 0.3 mV to -1.8 mV [15]. The zeta potential value in the range -30 mV to +30 mV indicates stability of formulation batch and is mentioned in **Table 4.**

TABLE 4: ZETA POTENTIAL OF VARIOUS BATCHES OF SLN

SR. NO.	FORMULATION CODE	ZETA POTENTIAL (MV)
1	PF1	-1.8
2	PF2	-0.4
3	PF3	-0.1
4	PF4	1.7
5	PF5	0.3

The zeta potential graphs of formulation batches PF1, PF2, PF3, PF4 and PF5 are represented in Figure 8, 9, 10, 11 and 12.

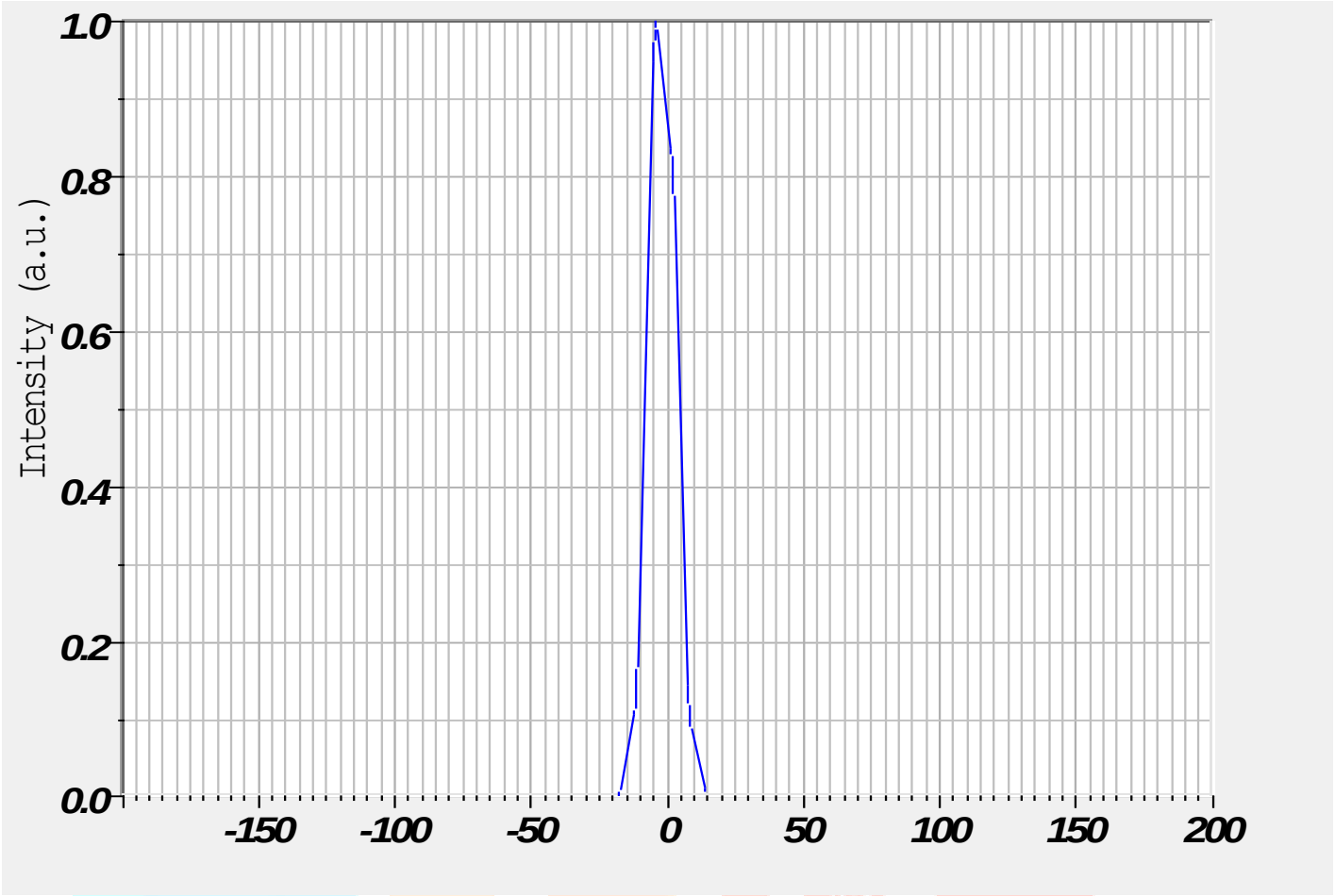

Figure 8: Zeta potential of formulation PF1

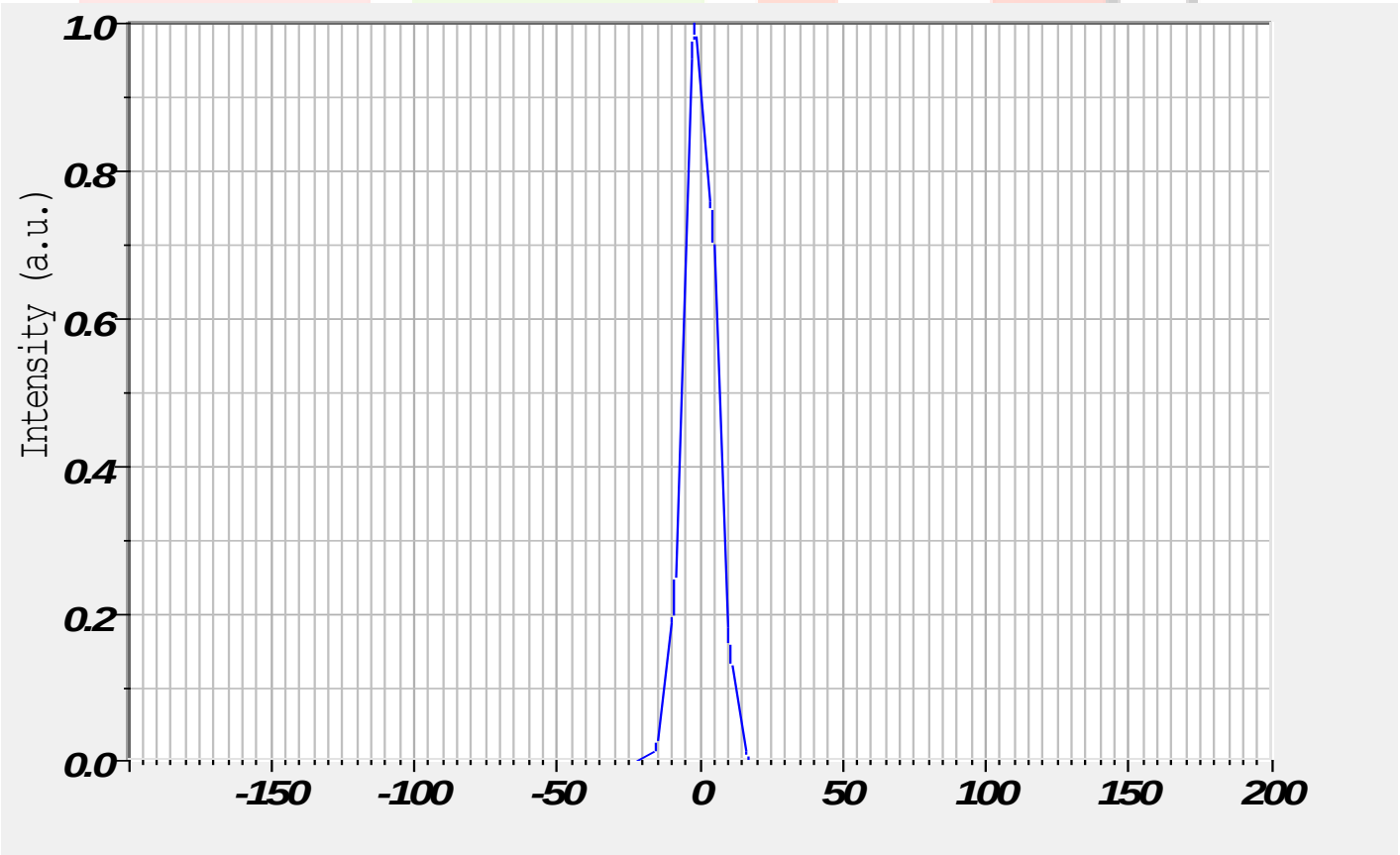

Figure 9: Zeta potential of formulation PF2

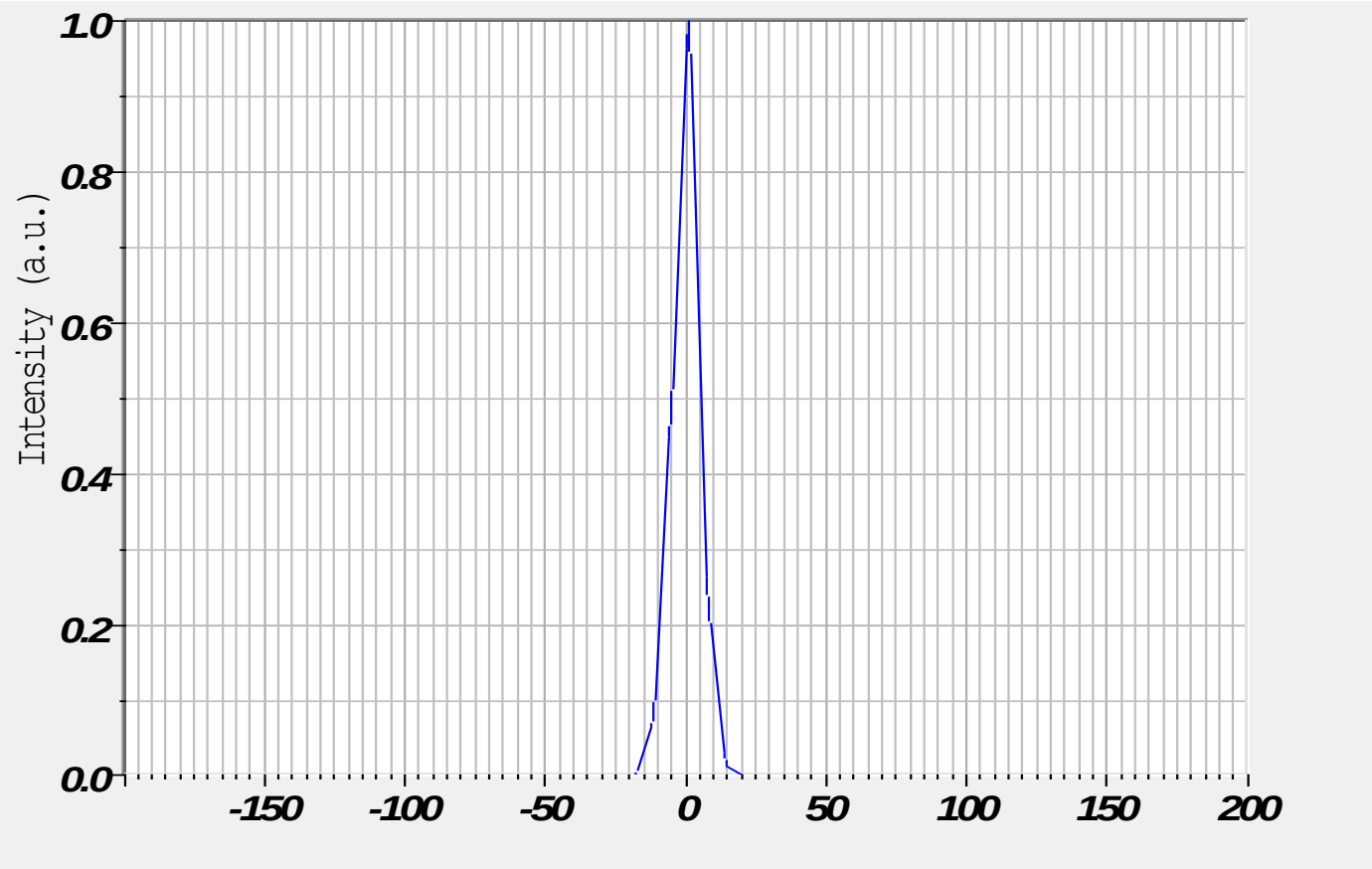

Figure 10: Zeta potential of formulation PF3

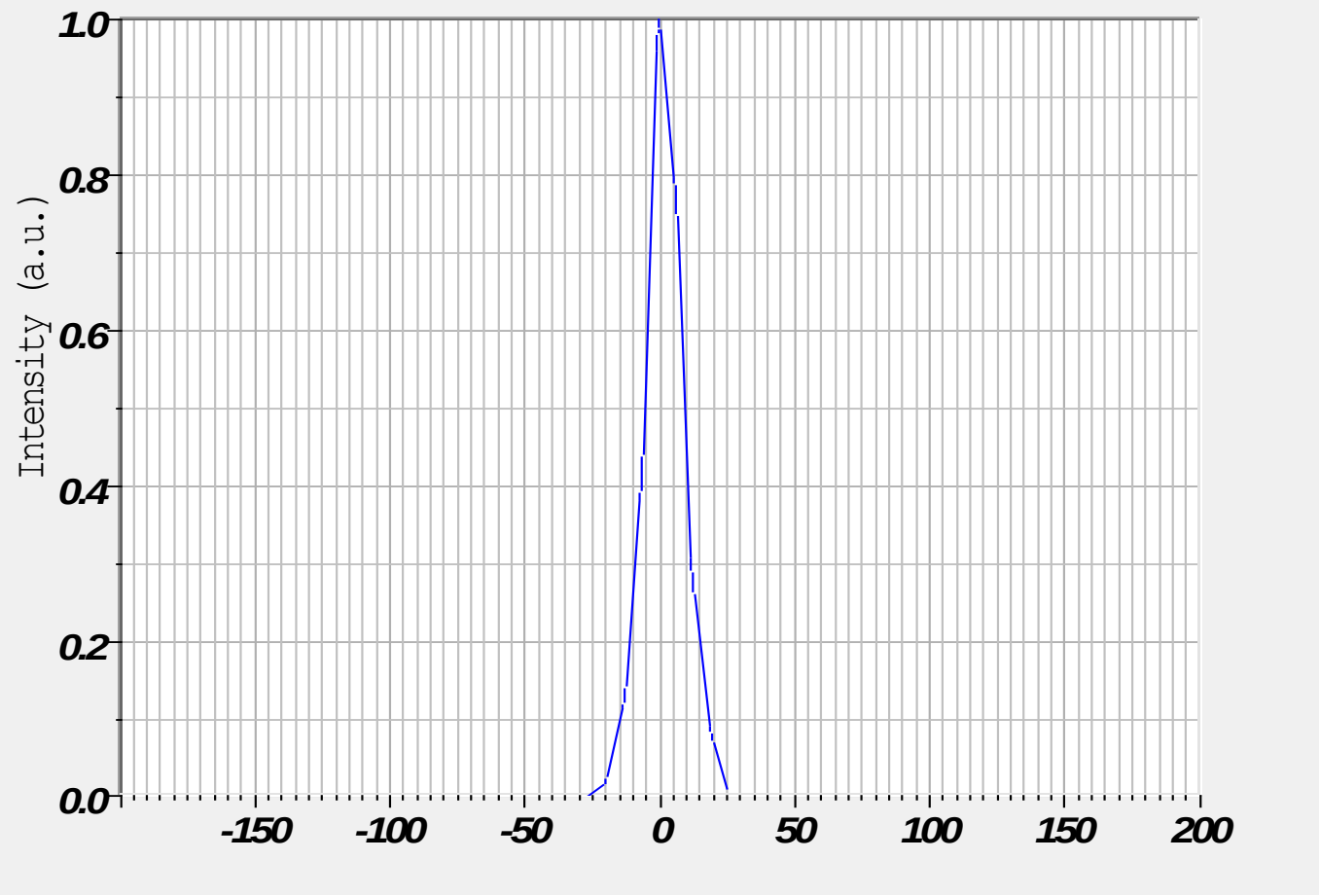

Figure 11: Zeta potential of formulation PF4

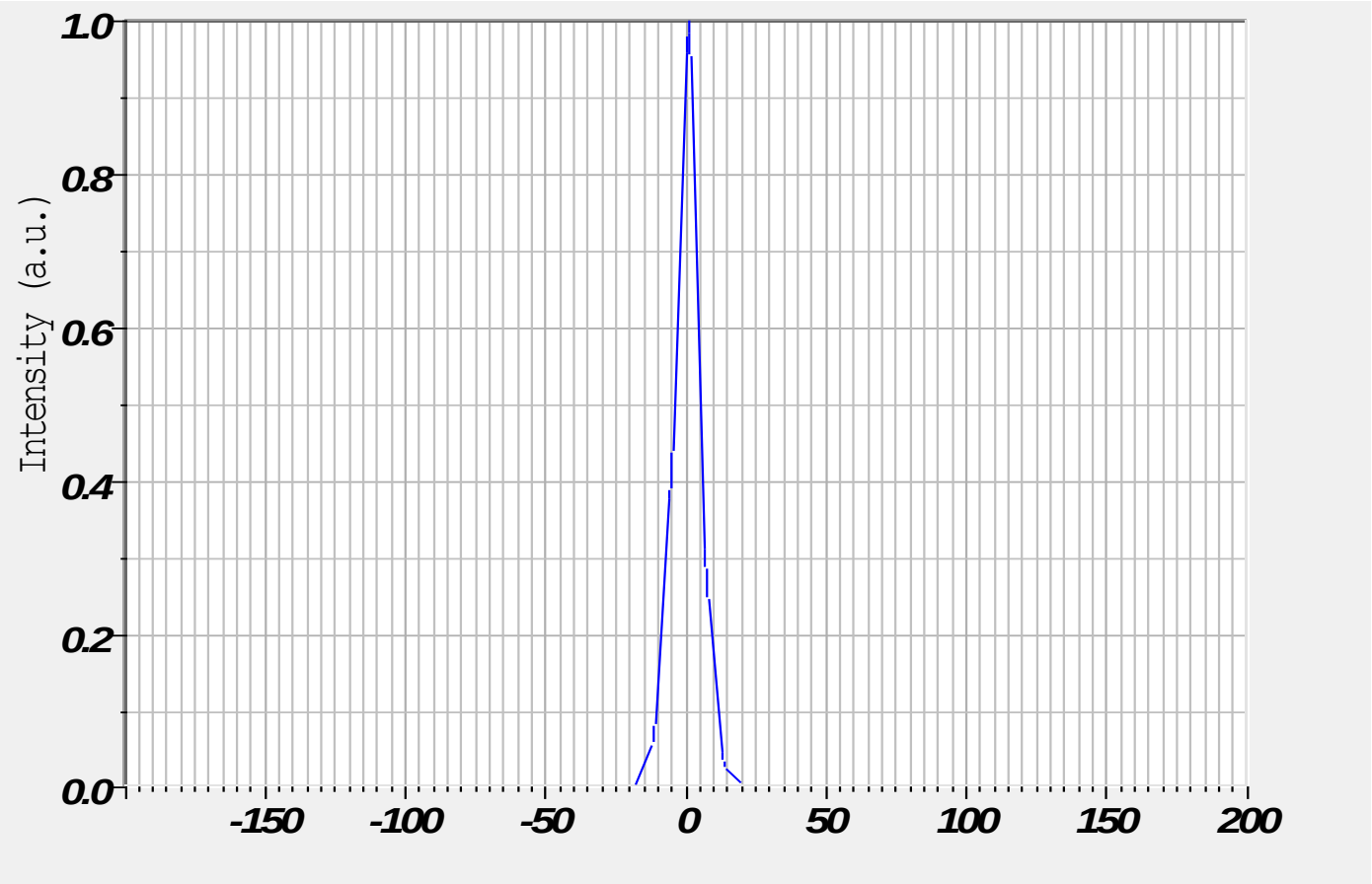

Figure 12: Zeta potential of formulation PF5

VIII. PERCENT ENCAPSULATION EFFICIENCY AND LOADING CAPACITY

The percent encapsulation efficiency (%EE) and percent drug-loaded (%DL) of the resulting SLN is mentioned in **Table 5 and 6.**

TABLE 5: DRUG LOADING (%) AND ENCAPSULATION EFFICIENCY (%) OF DIFFERENT BATCHES CONTAINING ARTEMETHER

SR.NO.	FORMULATION BATCH CODE	DRUG LOADING (%)	ENCAPSULATION EFFICIENCY (%)
1	PF1	6.36 ± 0.26	98.45± 0.11
2	PF2	4.31 ± 0.15	97.84 ± 0.67
3	PF3	3.25 ± 0.46	97.49 ± 0.23
4	PF4	2. 22 ± 0.37	96.89 ± 0.73
5	PF5	4.12± 0.62	95.39 ± 0.21

(Mean ± S.D, n = 3)

TABLE 6: % DRUG LOADING AND % ENCAPSULATION EFFICIENCY OF DIFFERENT BATCHES CONTAINING LUMEFANTRINE

SR.NO.	FORMULATION BATCH CODE	DRUG LOADING (%)	ENCAPSULATION EFFICIENCY (%)
1	PF1	2 92 ± 0.13	93.36 ± 0.10
2	PF2	4.22 ± 0.15	93.93± 0.59
3	PF3	3.62 ± 0.46	92.45 ± 0.42
4	PF4	3.52 ± 0.37	92.54 ± 0.78
5	PF5	4.00 ± 0.43	91.45 ± 0.23

(Mean ± S.D, n = 3)

The encapsulation efficiency of Artemether and Lumefantrine in the nanoparticles was found to be above 90 %. This indicated that 90 % of the Artemether and Lumefantrine were encapsulated in the SLN'S system while the remaining drug might be entrapped in the surfactant micelles. The solubilized drug would help in giving quick availability of Artemether and Lumefantrine in the body whereas encapsulated drug would be released in a sustained manner which may help in the prevention of recrudescence. Formulation PF1 was selected due to low particle size and high % Encapsulation efficiency [9].

IX. LYOPHILIZATION OF SLN'S OF ARTEMETHER AND LUMEFANTRINE

In most formulations the particle size was found to increase within a short period of time and hence lyophilization is a way to increase the stability of SLNs. Ostwald ripening as well as hydrolysis can be avoided by lyophilization. Moreover it also makes SLNs feasible to be incorporated into various dosage forms such as tablets, capsules, pellets, parenteral redispersion, etc. The SLN'S were prepared as per the procedure and its various parameters were evaluated.

9.1 Evaluation of Lyophilized SLN'S

9.1.1 Particle size

Particle size of lyophilized SLN'S (LPF1) was found to be 157.6 nm. The lyophilized SLN formulation batch exhibited good redispersibility upon ultrasonication. The particle size of lyophilized drug was higher than SLN dispersion Lyophilization of the SLN dispersion increased the particle size but it remained in the nanometer size range and graphically represented in **Figure 13.**

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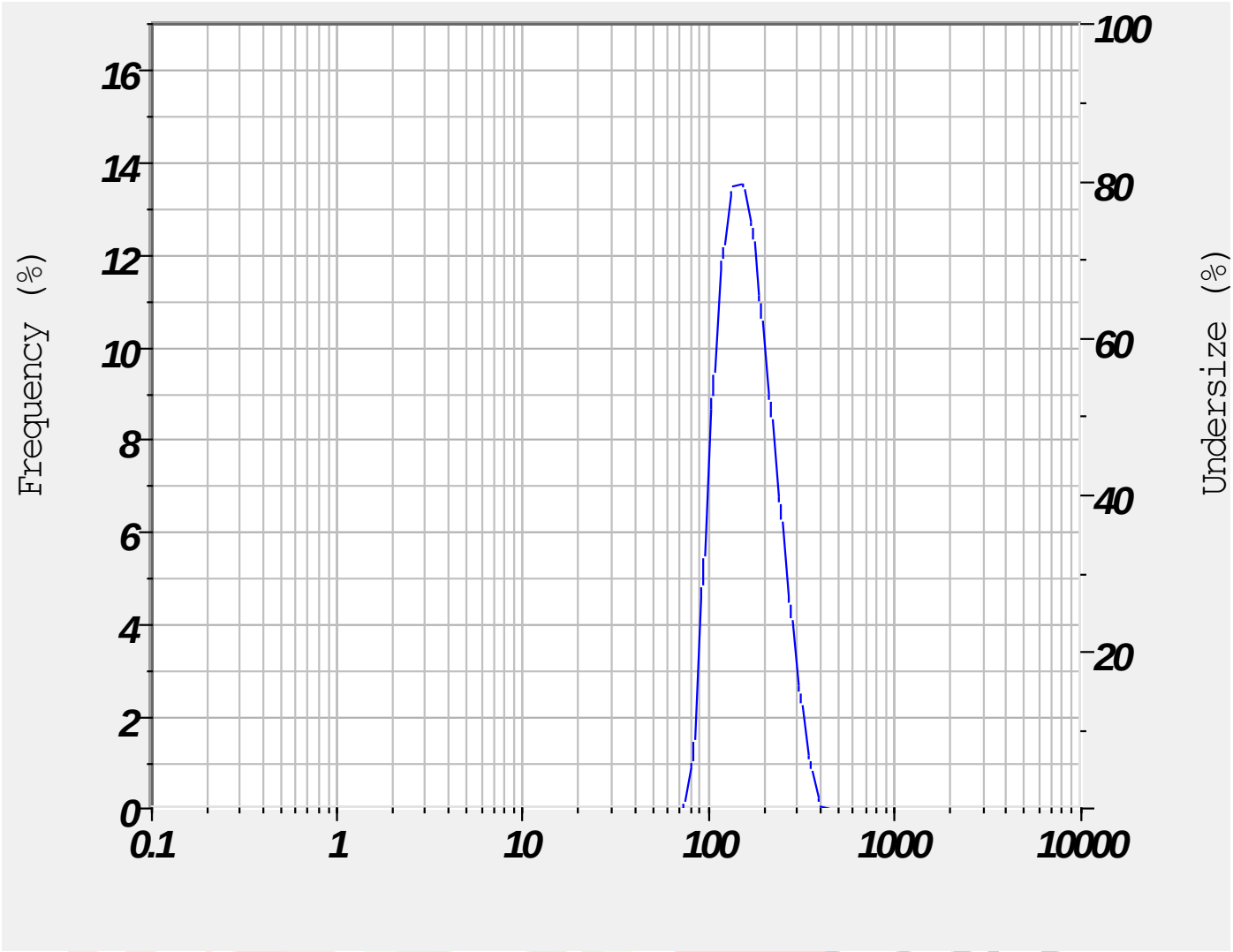

Figure 13: Particle size of lyophilized SLN'S

Zeta potential LPF1

The zeta potential value of SLN -0.2 mV indicated that the dispersion will remain in deflocculated state owing to its electrostatic repulsion between the particles and would be stable. The zeta potential of lyophilized nanoparticles indicates stability and non-agglomerating tendency of powder and the graph is depicted in **Figure 14.**

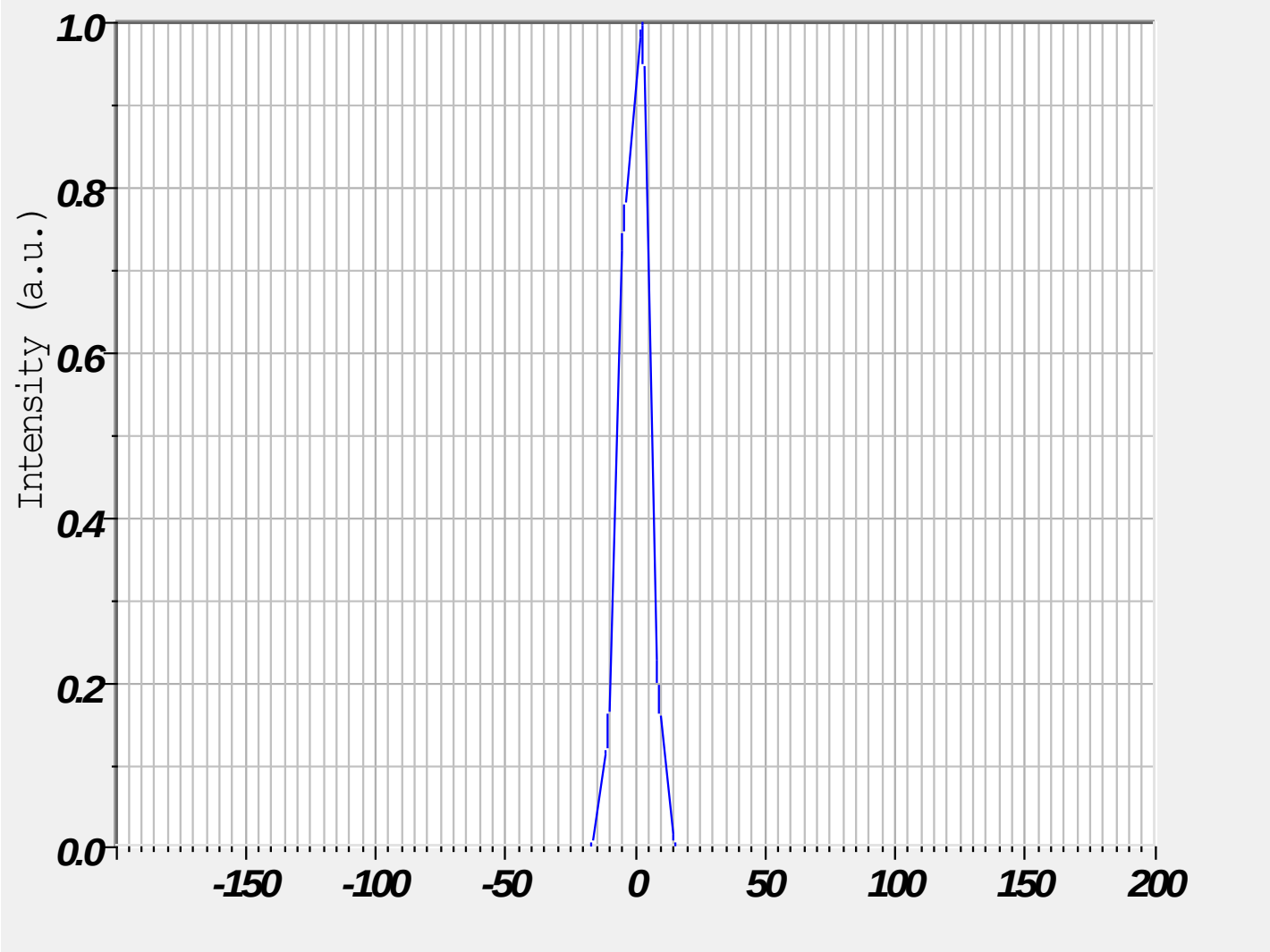

Figure 14: Zeta potential of Formulation LPF1

9.1.2 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%) and 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 15** and **Figure 16** respectively. The drug dissolution studies of SLN'S, marketed formulation (Lumerax®) and pure drug for Artemether and Lumefantrine is given in **Table 7** and **Table 8** respectively.

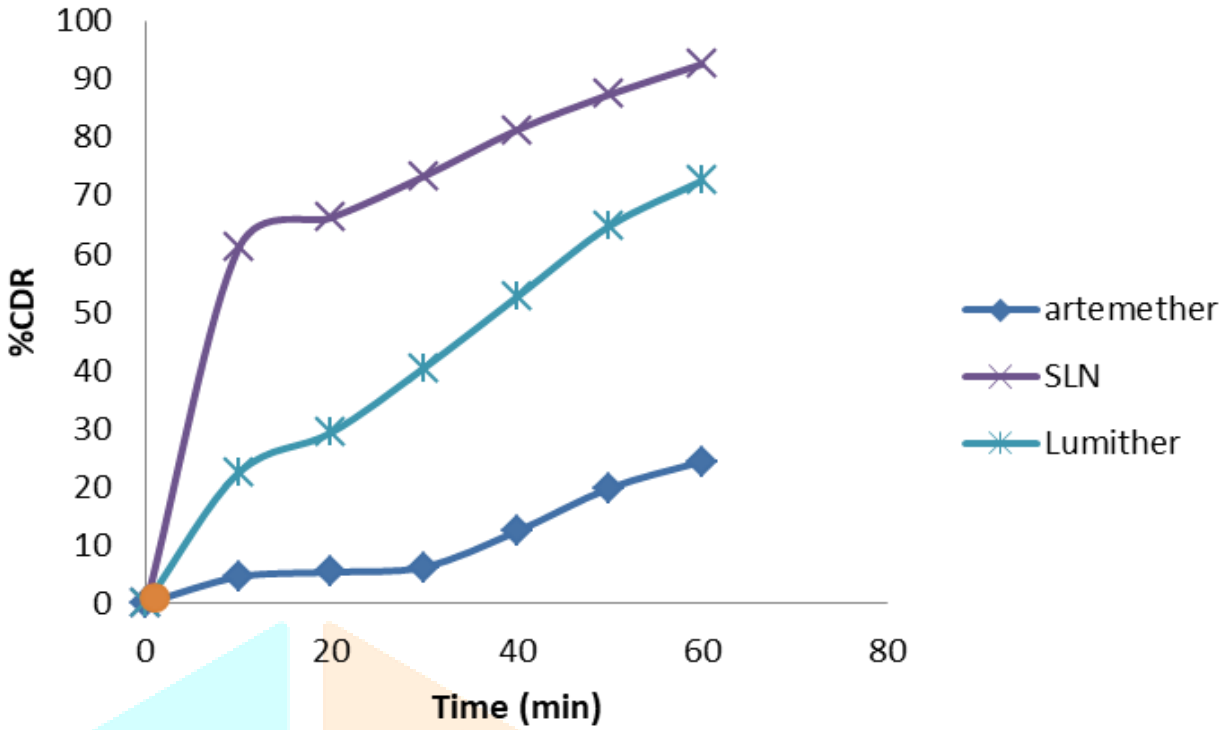

FIGURE 15: CUMULATIVE DRUG RELEASE OF SLN'S, MARKETED FORMULATION (LUMERAX®) AND PURE DRUG ARTEMETHER

TABLE 7: CUMULATIVE DRUG RELEASE OF SLN'S, MARKETED FORMULATION (LUMERAX®) AND PURE DRUG ARTEMETHER

SR.NO.	TIME IN MINUTES	% CDR PURE ARTEMETHER	% CDR SLN'S	% CDR LUMERAX ®
1	0	0	0	0
2	10	4.56±0.39	61.12±0.03	22.45±0.08
3	20	5.34±0.98	66.21±0.37	29.34±0.40
4	30	6.23±0.77	73.32±0.52	40.43±0.45
5	40	12.35±0.76	81.23±0.82	52.56±0.87
6	50	19.76±0.23	87.34±0.22	64.74±0.62
7	60	24.34±0.96	92.56±0.98	72.56±0.12

(Mean ± S.D, n = 3)

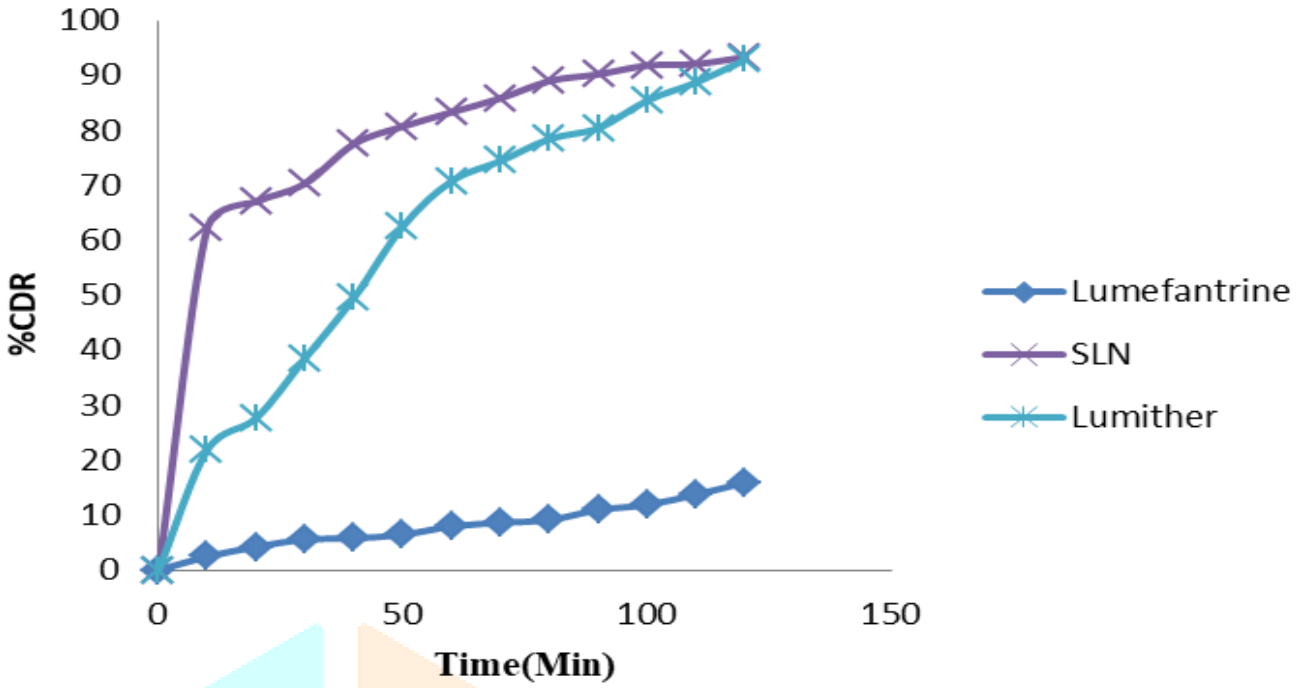

FIGURE 16: CUMULATIVE DRUG RELEASE OF SLN'S, MARKETED FORMULATION (LUMERAX®) AND PURE DRUG LUMEFANTRINE

TABLE 8: CUMULATIVE DRUG RELEASE OF SLN'S AND MARKETED FORMULATION AND PURE DRUG LUMEFANTRINE

SR. NO.	TIME IN MINUTES	% CDR PURE LUMEFANTRINE	% CDR SLN'S	% CDR LUMERAX ®
1	0	0	0	0
2	10	2.46±0.56	62.38±0.95	21.87±0.71
3	20	4.23±0.76	67.01±0.05	27.67±0.44
4	30	5.56±0.05	70.34±0.8	38.56±0.54
5	40	5.76±0.09	77.54±0.71	49.56±0.09
6	50	6.44±0.21	80.71±0.32	62.54±0.76
7	60	7.94±0.12	83.32±0.08	70.78±0.83
8	70	8.62±0.08	85.85±0.23	74.45±0.86
9	80	9.13±0.72	88.99±0.04	78.43±0.49
10	90	10.94±0.87	90.21±0.98	80.43±0.05
11	100	11.89±0.21	91.81±0.59	85.44±0.09
12	110	13.74±0.98	92.08±0.21	88.74±0.12
13	120	15.94±0.02	93.34±0.43	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[16] and may be due to the formation of hybrid surfactant sheathing the surface spherical shaped[17].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 [18] Thus, from dissolution studies it can be concluded that aqueous solubility and dissolution rate of prepared formulation batches was significantly enhanced.

X. CHARACTERIZATION OF OPTIMIZED FORMULATION

10.1. Differential Scanning Calorimetry

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 thermo gram 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. The Differential Scanning Calorigraph of Artemether and Lumefantrine are depicted in **Figure 17** and **Figure 18** respectively.

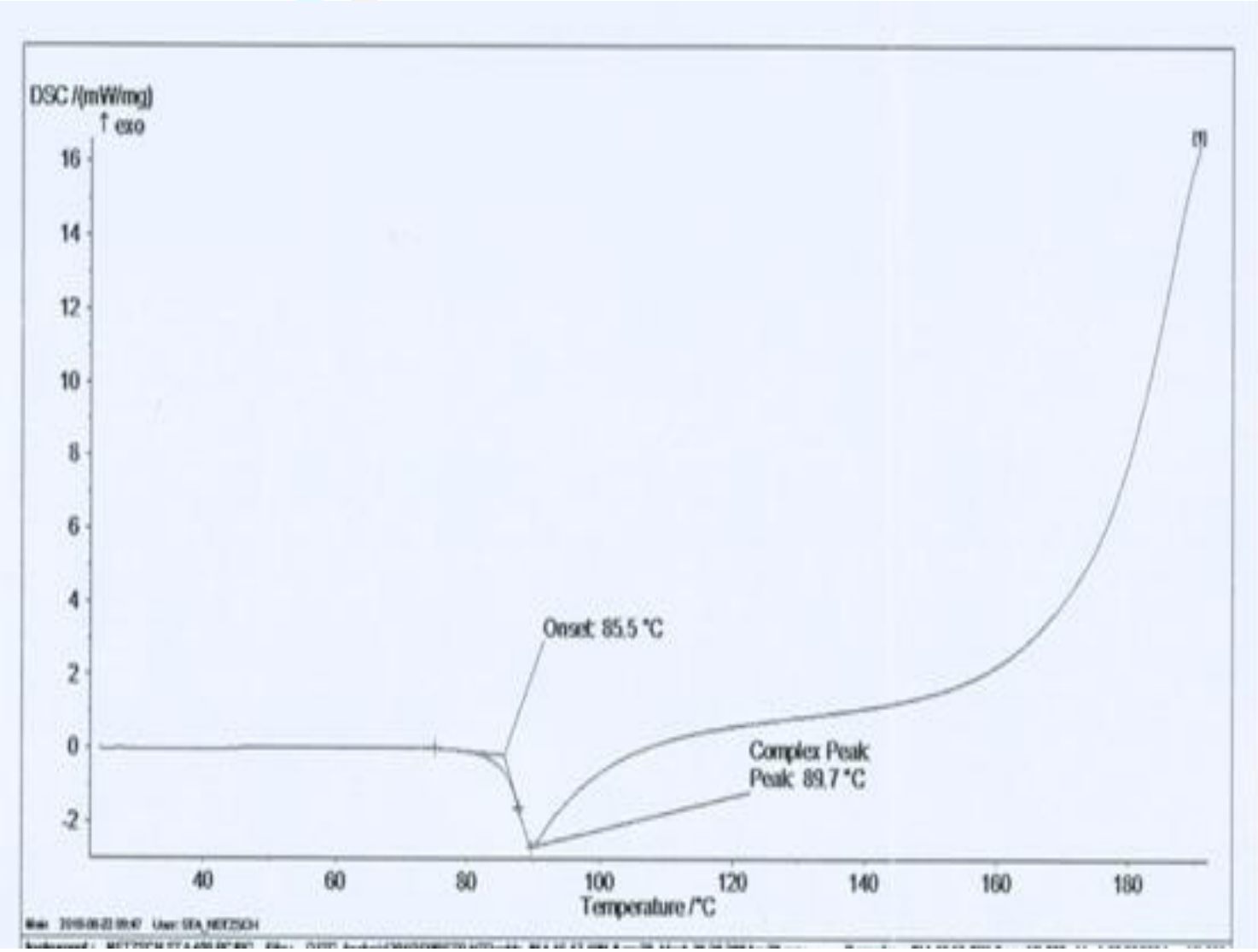

FIGURE 17: DIFFERENTIAL SCANNING CALORIGRAPH OF ARTEMETHER

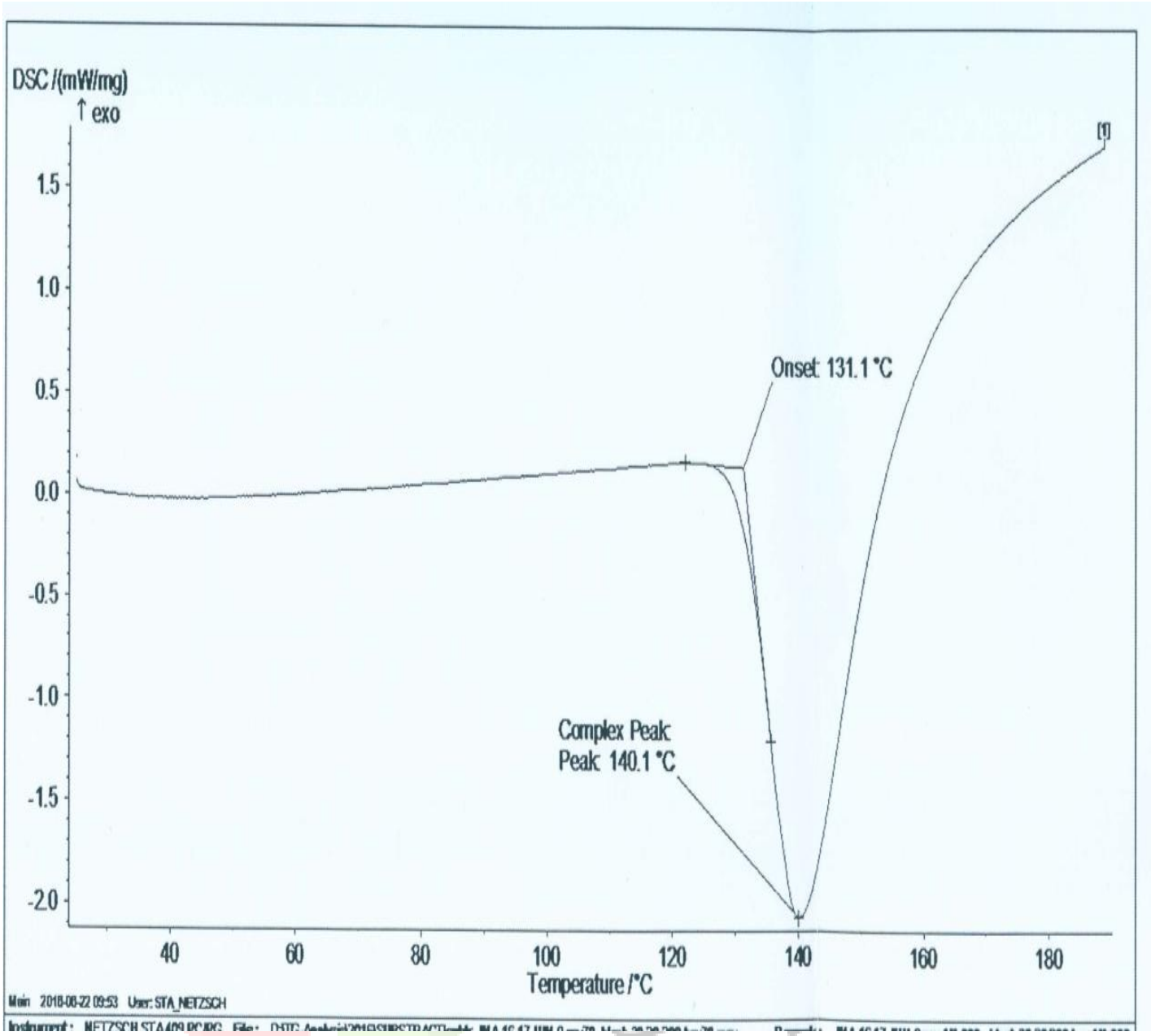

FIGURE 18: DIFFERENTIAL SCANNING CALORIGRAPH OF LUMEFANTRINE

Differential scanning Calorimetry of pure drugs Artemether and Lumefantrine represented sharp endotherm peak at their melting points and SLNS represented no such peak which indicated change in melting behavior of drug and retention of crystallization. The disappearance of the melting endotherm in the DSC scan of SLNS 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 of SLN is given in **Figure 19.**

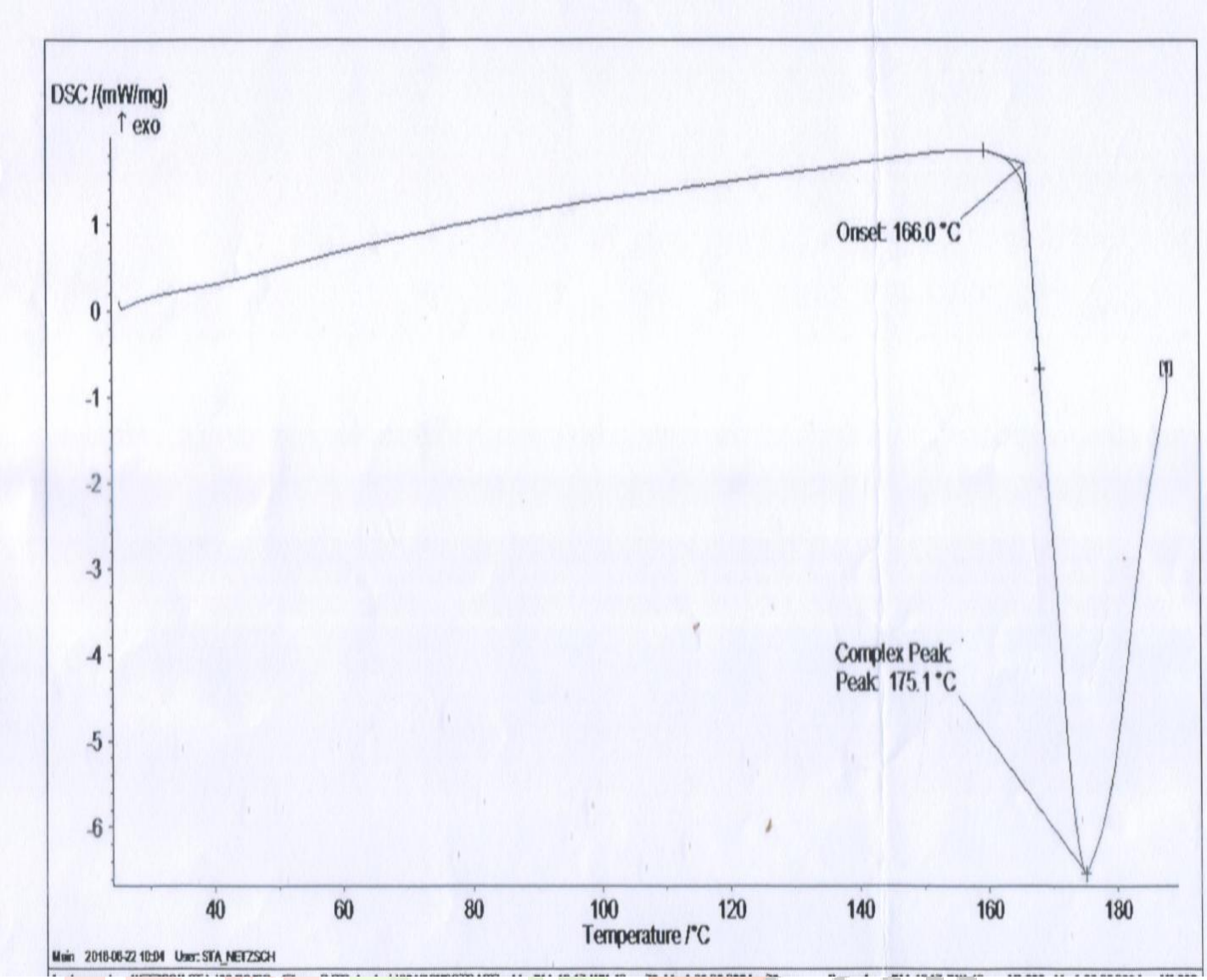

FIGURE 19: DIFFERENTIAL SCANNING CALORIGRAPH OF SLN

10.2. 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 SLNS. 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 20** and **Figure 21** respectively.

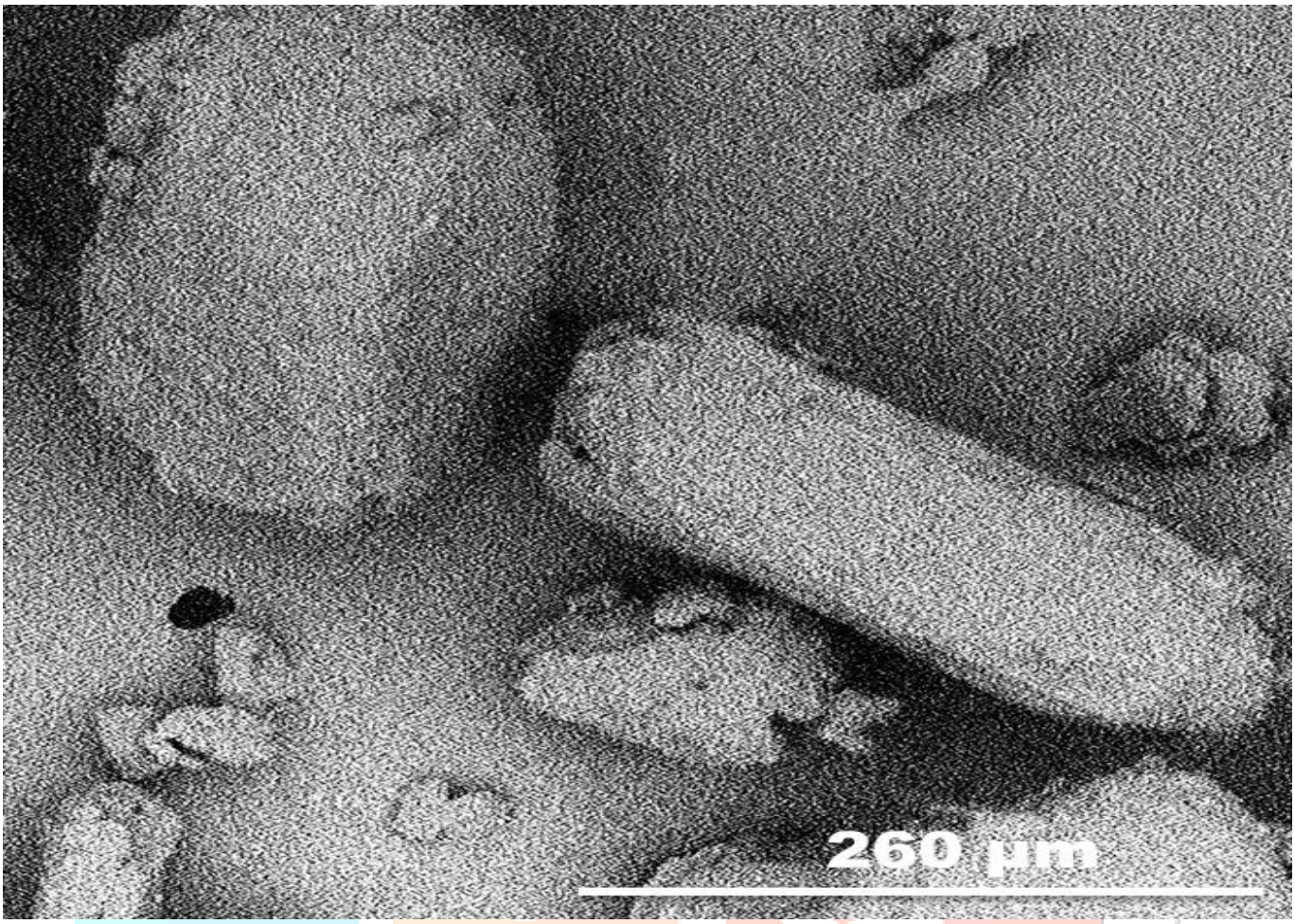

FIGURE 20: SCANNING ELECTRON MICROGRAPH OF ARTEMETHER

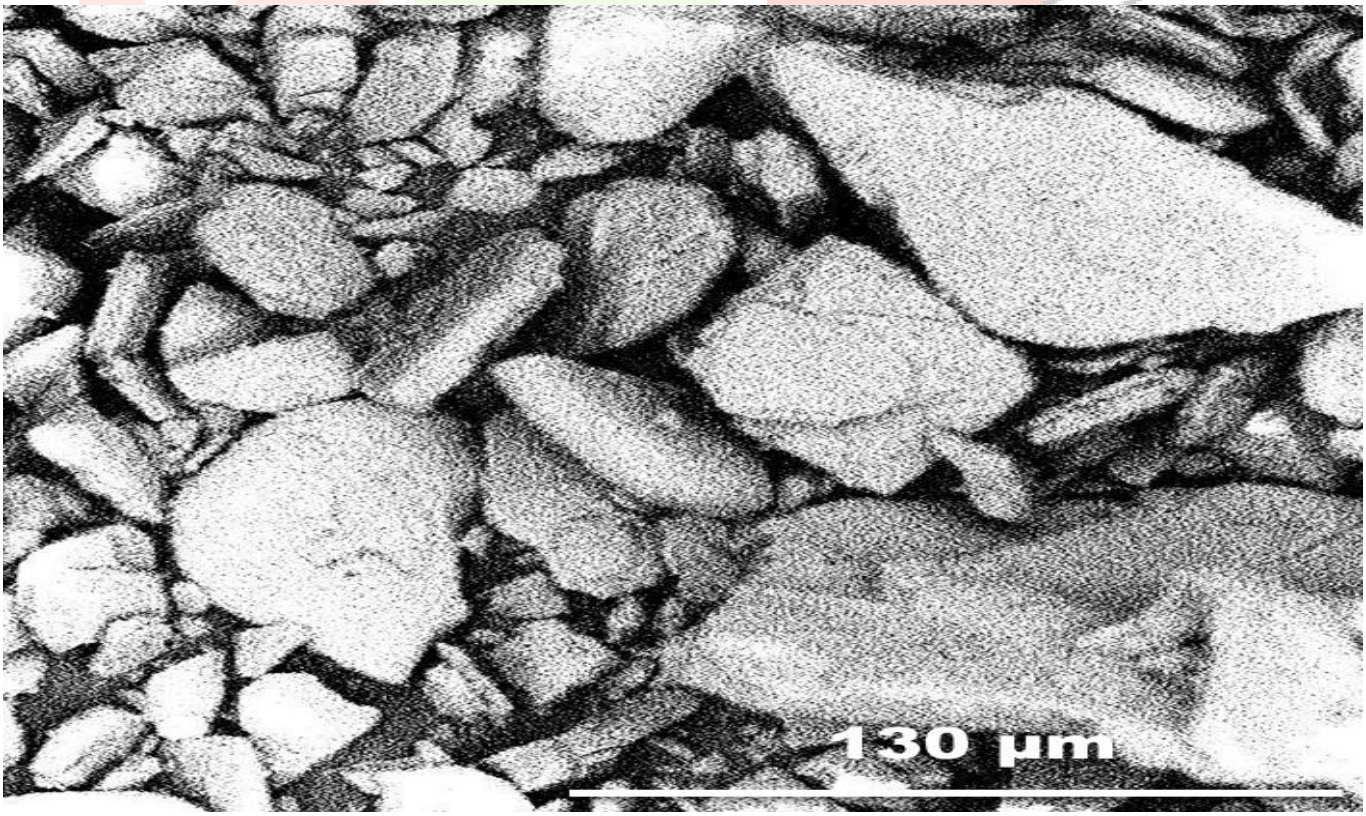

FIGURE 21: SCANNING ELECTRON MICROGRAPH OF LUMEFANTRINE

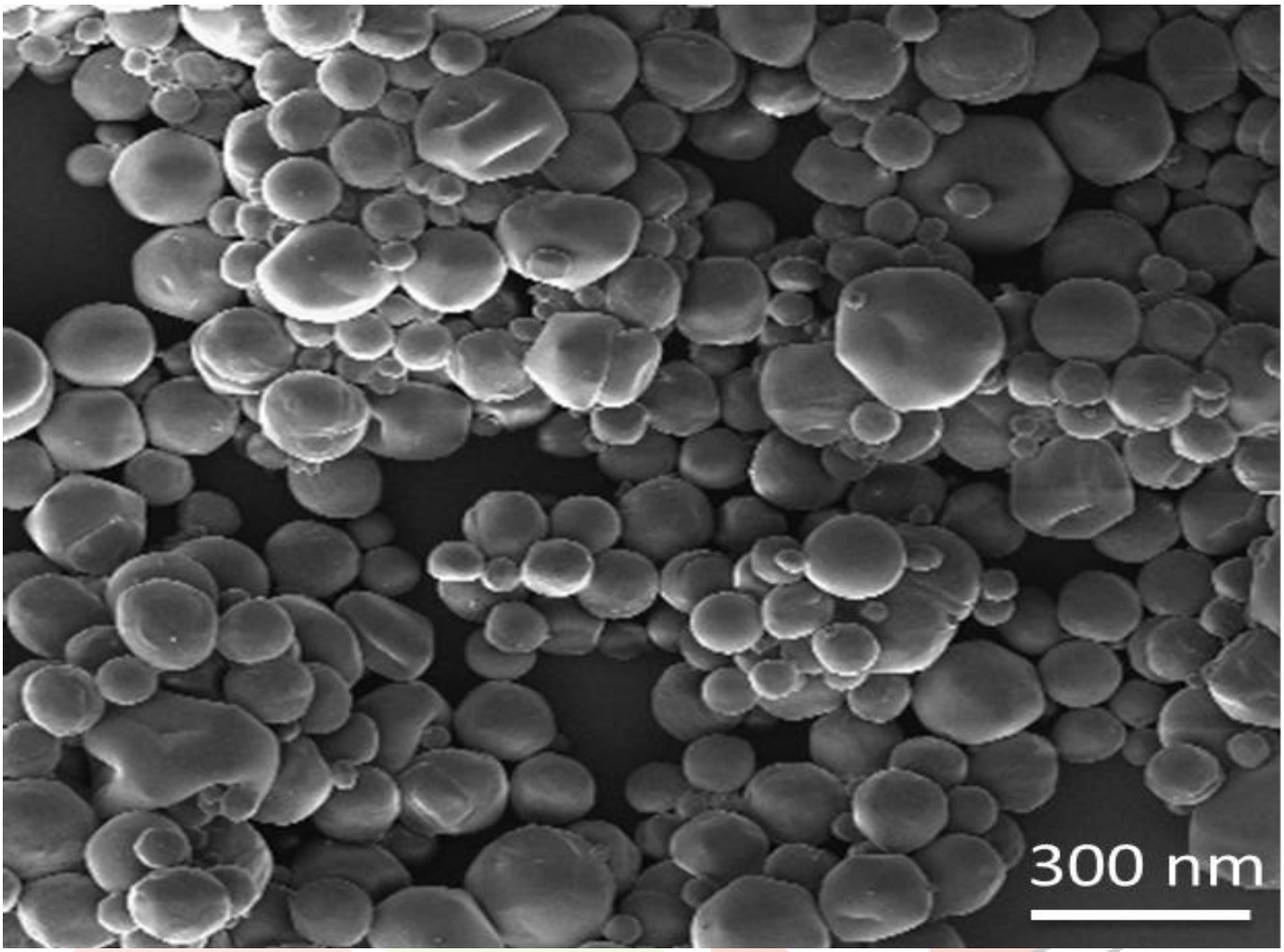

FIGURE 22: SCANNING ELECTRON MICROGRAPH OF SLN'S

Pure drugs appeared under the scanning electron microscope having rough surfaces and crystalline forms. However the SEM of its SLN'S indicated that all the particles were found to be roughly spherical in shape with a well-defined periphery. The SLN indicated no agglomeration in SEM image due to Stabilizer in coating surface and further giving small particle size. The surface morphology of SLN indicated that intact nature of SLN would aid enhanced solubilisation. Scanning Electron Micrograph of SLN is presented in **Figure 22.**

10.3. Infrared spectroscopy

The FTIR spectrum of Artemether revealed the presence of major functional groups present in structure of Artemether supporting is identity and shown in **Figure 23** and major spectral characteristics are given in **Table 9.**

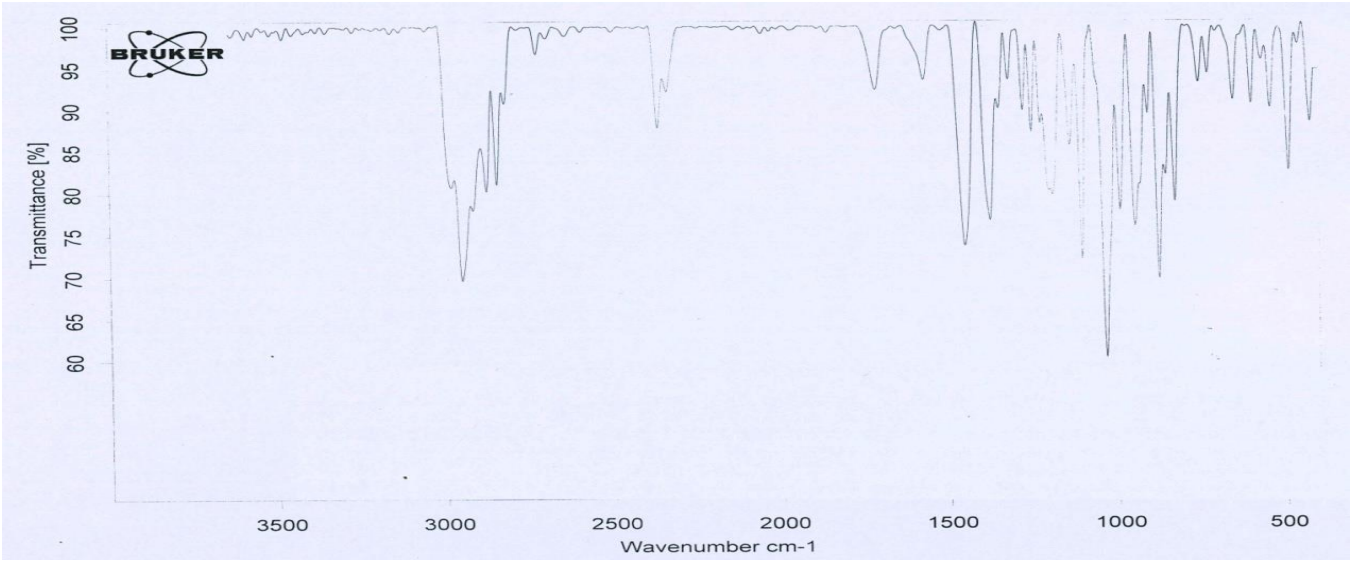

FIGURE 23: INFRARED SPECTRUM OF ARTEMETHER

TABLE 9: 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

IR spectrum of Lumefantrine revealed the presence of major functional groups present in the structure of Lumefantrine supporting its identity and shown in **Figure 24** and its interpretation is mentioned in **Table 10.**

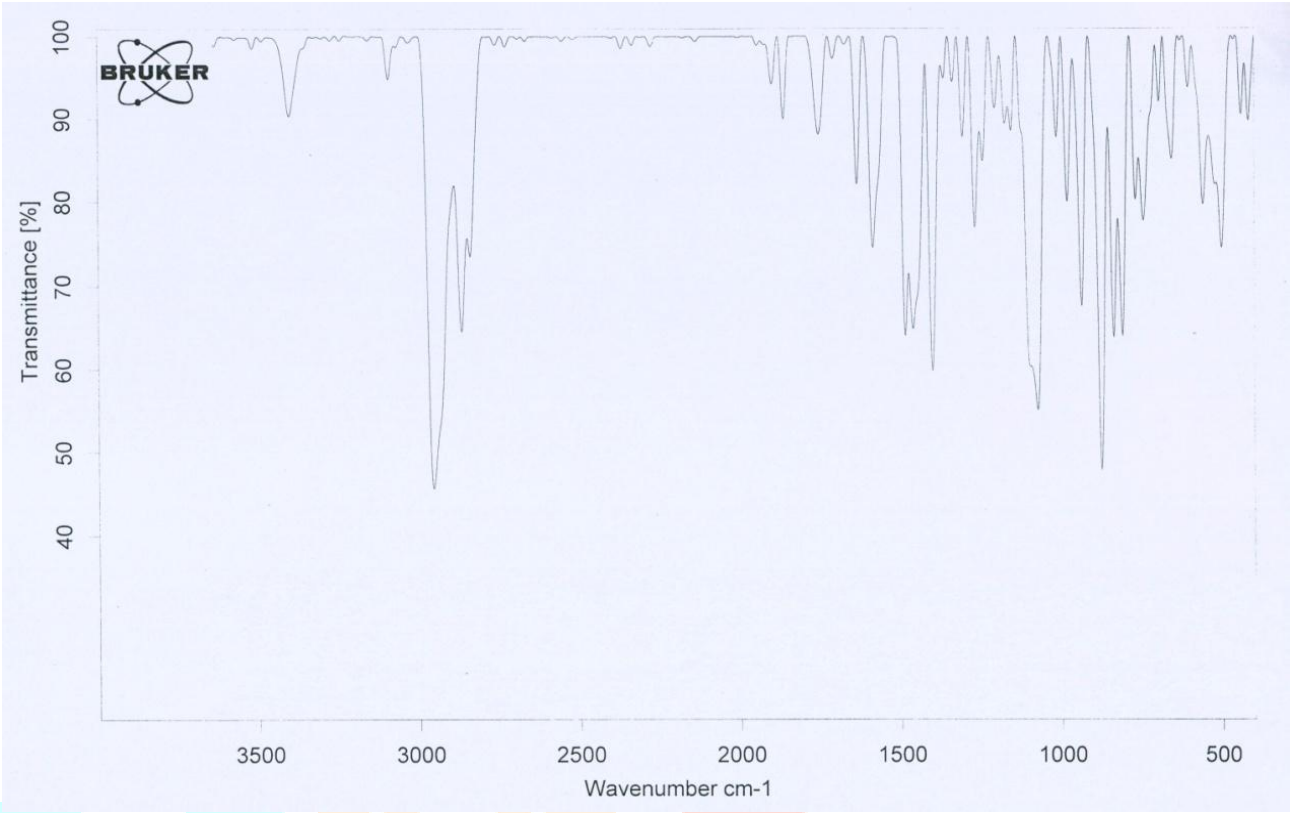

FIGURE 24: INFRARED SPECTRUM OF LUMEFANTRINE

TABLE 10: 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

FTIR was used to study the drug excipient compatibility. FTIR spectra of SLN 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 SLNS of Artemether and Lumefantrine is mentioned in **Table 11** respectively. Infrared spectrum of SLNS is shown in **Figure 25.**

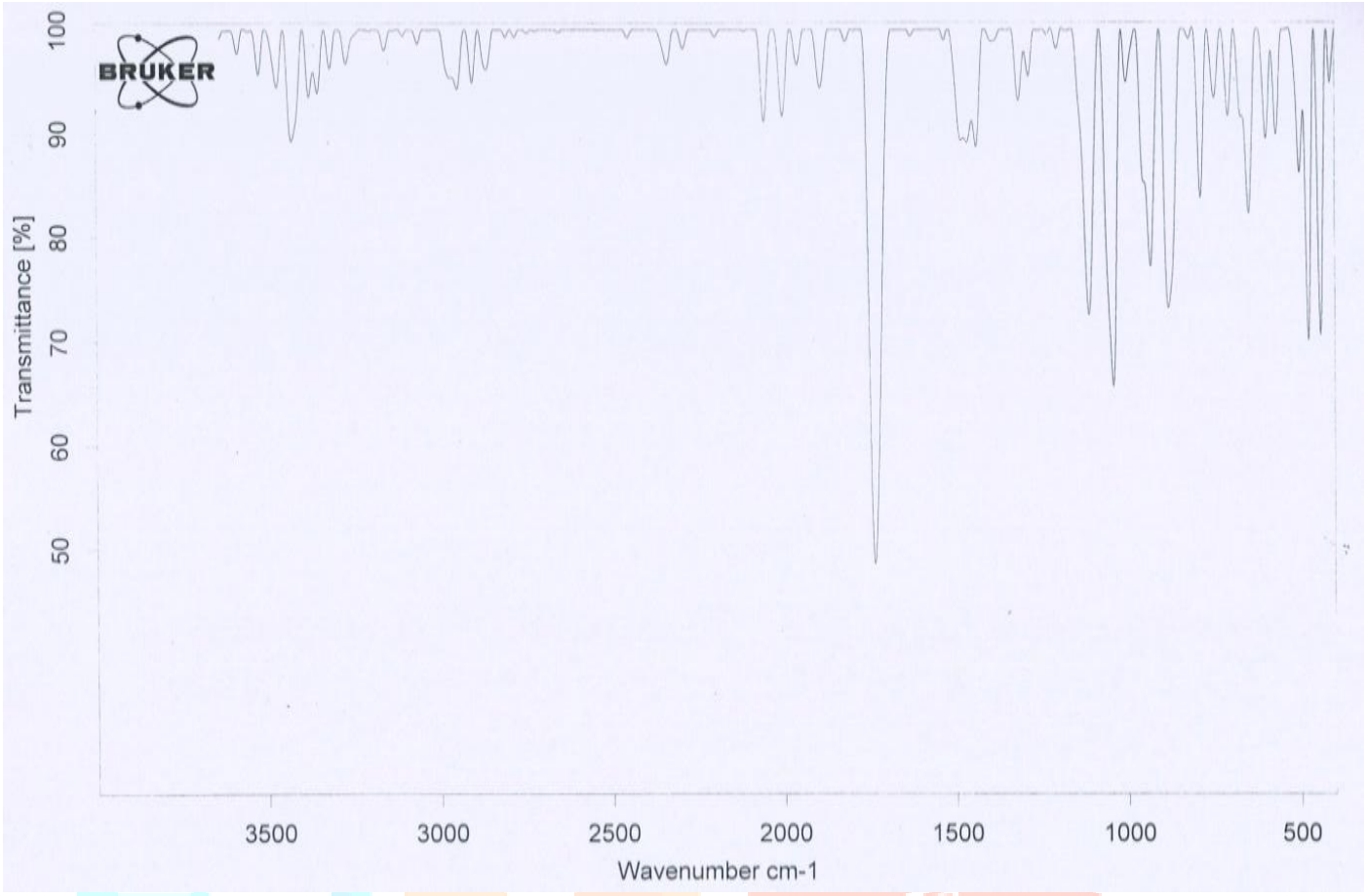

FIGURE 25: INFRARED SPECTRUM OF SLN'S

TABLE 11: INTERPRETATION OF IR SPECTRUM OF SLN

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

10.4. X- ray Diffraction

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 X ray diffraction pattern of Artemether is depicted in **Figure 26.**

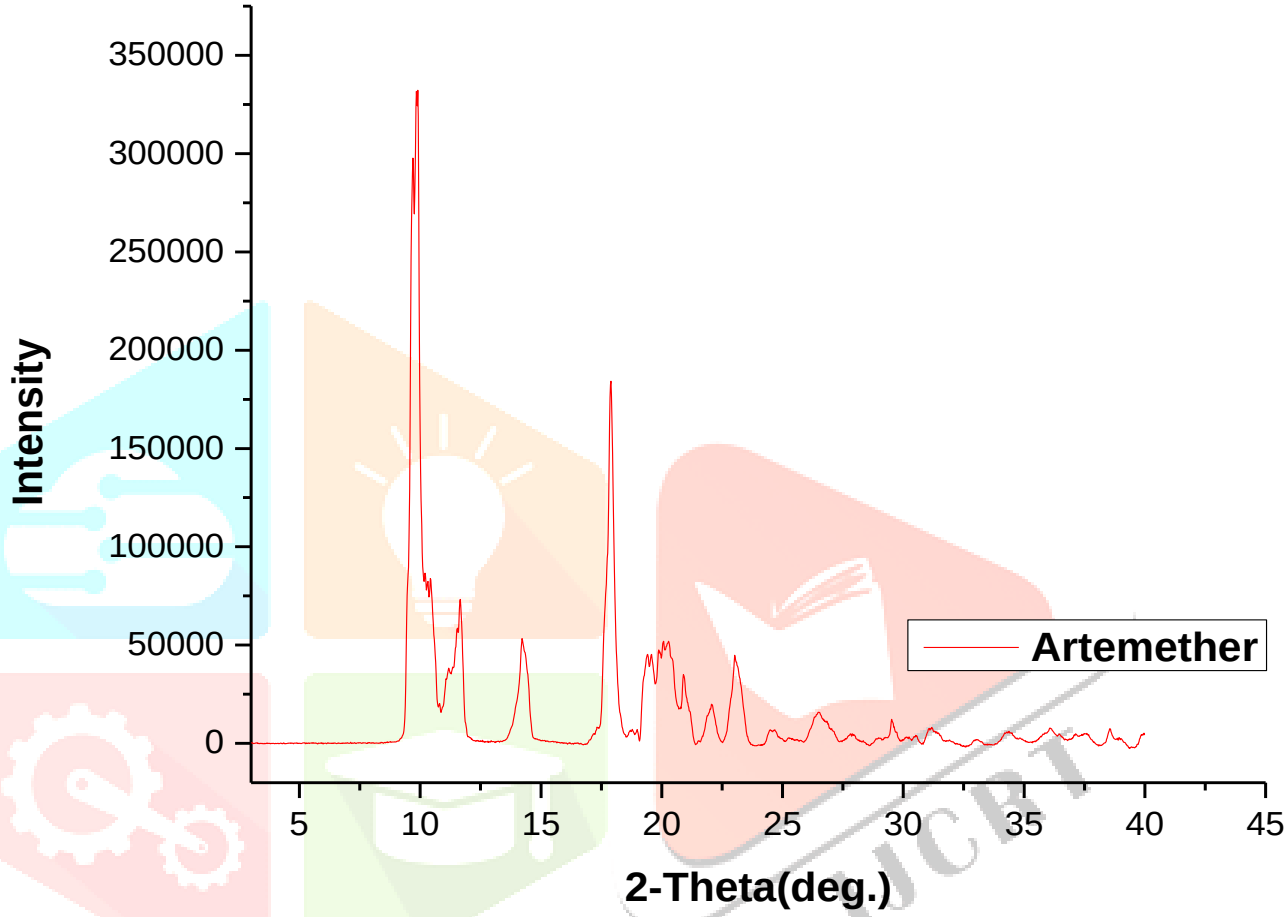

FIGURE 26: X-RAY DIFFRACTION PATTERN OF ARTEMETHER

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. The X-ray diffraction pattern of Lumefantrine is mentioned in **Figure 27.**

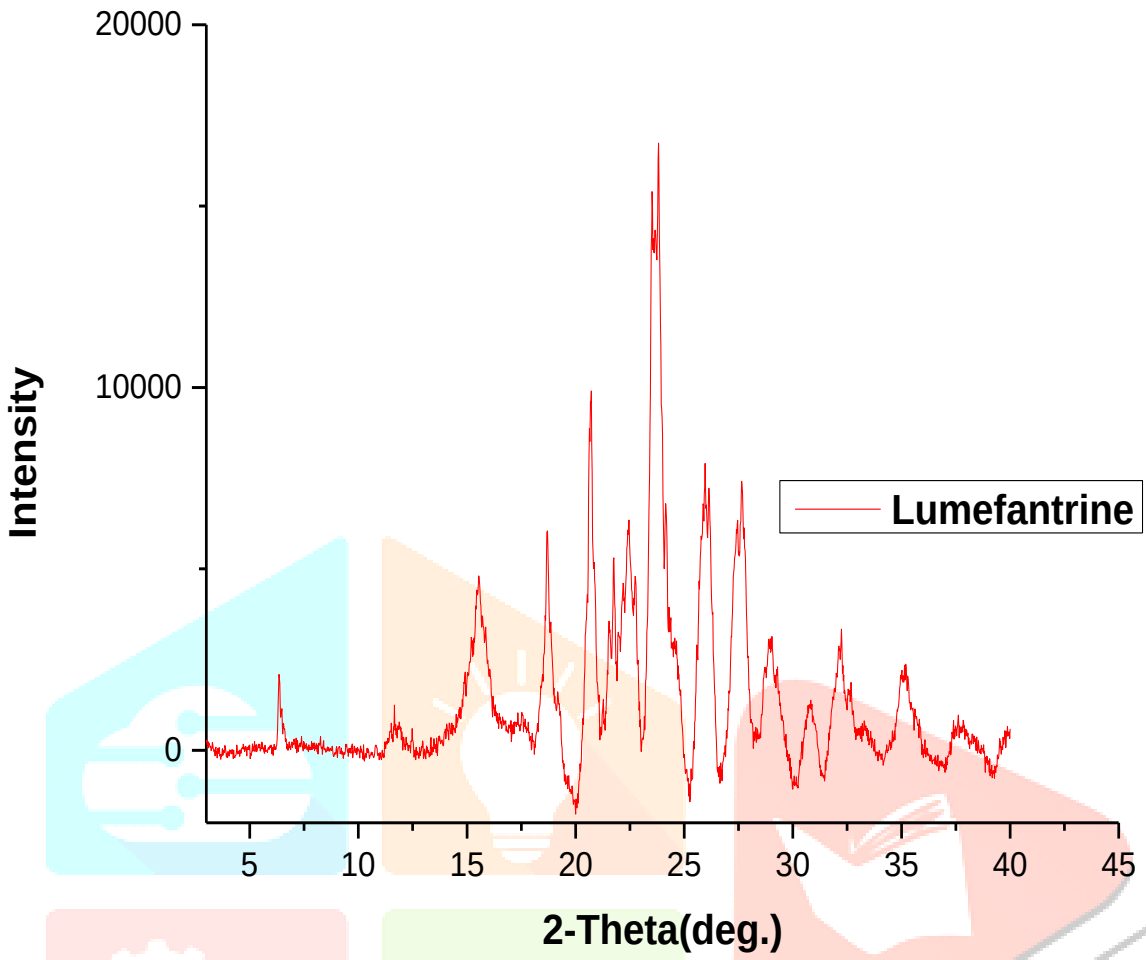

FIGURE 27: X-RAY DIFFRACTION PATTERN OF LUMEFANTRINE

X-ray diffraction pattern of SLNS of Artemether and Lumefantrine verified the crystal transformation pattern of the drug in the SLN. 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 SLNS is depicted in **Figure 28.**

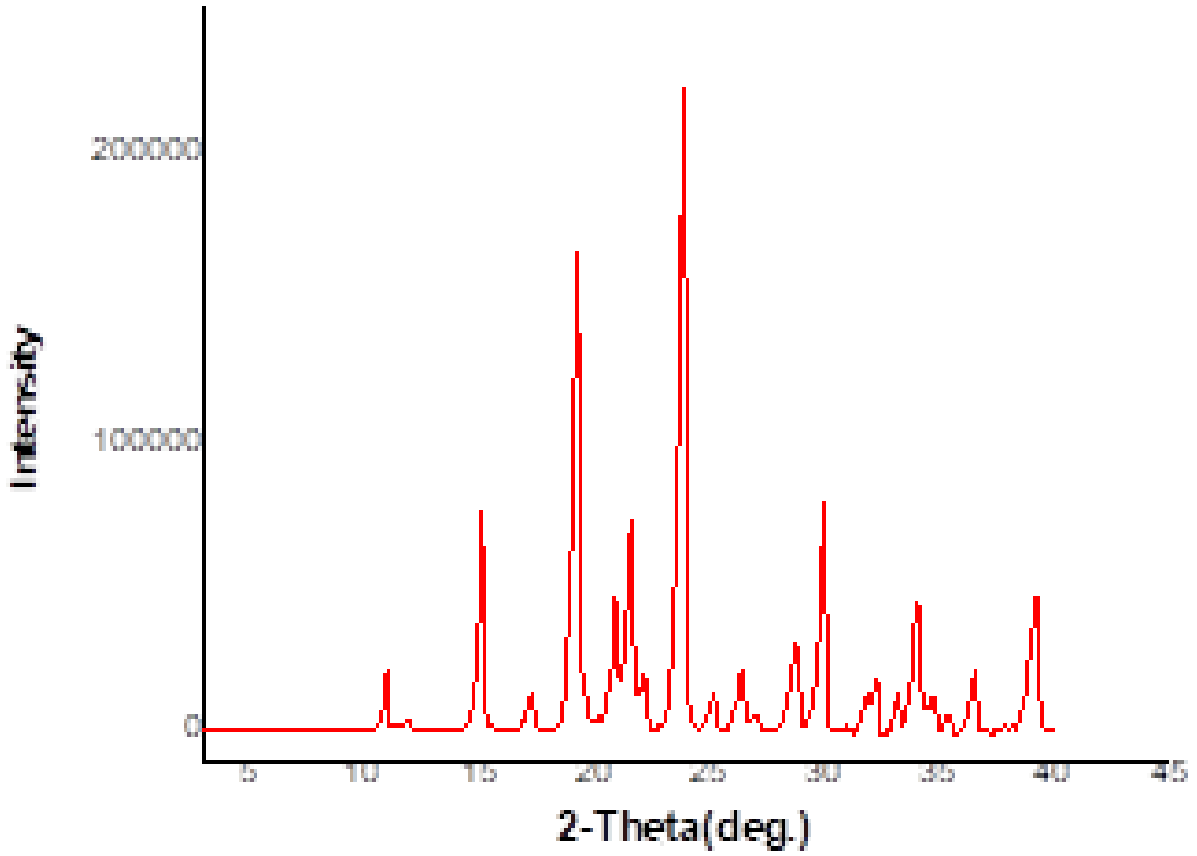

Figure 28: X-ray diffraction pattern of SLN'S of Artemether and Lumefantrine

XI. 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 SLN 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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