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"Formulation and Development of Proniosomal Drug Delivery System for Brain Targeting"

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Abstract:

Cerebral malaria is a major public health concern and neurological complication of *Plasmodium falciparum* infection. The cerebral malaria is infected to RBC, Platelets, and leukocytes and as an effect on the central nervous system the patient was suffering from coma, unconsciousness, and death.

Artemether contain artemisinin effective against *Plasmodium falciparum*. These artemether is isolated from the plant Artemisia annua and its derivatives artemisinin is approved for the treatment of Novel anticancer agent against some colon cancer, melanoma, and leukemia and breast cancer. Artemether is also used as a management of cerebral malaria (CM). It is in the Bcs Class IV having poor aqueous solubility and short half life 3 to 5 hrs. The ARM having an intramuscular injection for the treatment of the cerebral malaria but it is having a more pain and the patient compliance was poor so that im route is not suited for administration of ARM for treatment of CM.

Hence to overcome this drawback to investigate alternative delivery for ARM nose to brain delivery. The conventional route for this drug delivery is not suited for cerebral malaria, because drug releases into systemic circulation and it not gives a drug effect on brain. So it is not useful for treating CM. Therefore this is a need of nose to brain drug delivery is effective to deliver ARM for CM.

In recent study Proniosomes are prepared by using 3 different carriers for optimum size, with maximum drug entrapment efficiency (%EE), and drug release. We using a specific carrier Neusilin (NUS2, NUFL2) and Surface modified lactose. Surface modification is effective to alter adhesion/cohesion and to improved aerosol performance and affect the particle adhesion and reduced

contact area of lactose. Neusilin is used for drying and improved the flow properties of powder to prepare a Proniosomes.

Keywords: Cerebral malaria, Neurological, Intramuscular, Adhesion Aerosol.

Introduction:

The field of nanotechnology research has shown a great progress in the developing of novel nanocarrier as controlled drug delivery systems. Proniosomes technology is started two decades ago and used as a stable precursor than Niosome. Numerous research articles have been published in the study of the Proniosomes formulation for controlled drug delivery system. However, survey and discussion on the recent, rapidly growing reported studies along with their theoretical principal is required for the fully understanding and exploring the great potential of this approach of the Proniosomes for nasal drug delivery system. Hereby, we have been provided an illustrated and comprehensive study with aim of a development of nanotechnology based Proniosomes. First, physicochemical properties of Proniosome forming non-ionic surfactants and additive agents have been discussed. Second, a systematic survey of Proniosome preparation and Drug loading methods, administration routes, characterization of Proniosomes, their toxicity studies and mechanism of drug release; used in recent articles have been performed**.**

Niosomal drug delivery system

Niosome is the multilamellar vesicular structure of non-ionic surfactant. Niosomes are vesicles composed mainly of hydrated non-ionic surfactants and, cholesterol (CHOL) or its derivatives. The unique structures of Niosome make it capable of encapsulating both hydrophilic and lipophilic substances. This can be done by entrapping hydrophilic in vesicular aqueous core or lipophilic substances are encapsulated by their partitioning into the lipophilic domain of the bilayers. Thin lipid films or lipid cakes are hydrated of liquid crystalline bilayers become fluid, swell and form liposome. Agitation makes the hydrated lipid sheets detach and self-associate to form vesicles, preventing interaction of water with the hydrocarbon core of the bilayers at the edges so it forms a Proniosomes. [1]

The Proniosomes are also better than the Niosomes. [2] Proniosomal drug delivery system: Proniosomes have distinct advantages over conventional drug delivery system because the particles can act as drug reservoir system, in composition of the surfactant and cholesterol to have the affinity to target site.[3] However, there remain significant problems arising in the liposome's and Niosome for drug delivery liposome's have problem associate with degradation by hydrolysis or oxidation and sedimentation, aggregation or fusion and other problems are sterilization, large scale production and storage problem because of chemical and physical stability. [4, 5]

The large-scale production of Proniosomes does not require any special conditions, unacceptable solvents or precautions. It is dry, free-flowing formulations of surfactant-coated carrier, which can be rehydrated by brief agitation in hot water or buffer solution to form a multi-lamellar Niosome suspension suitable for administration by oral or other routes for administration.[6] These versatile delivery systems have potential to be used as a carrier, a wide range of active compounds. [7]

Components of Niosomes: Niosomes mainly contains following types of components:

Non-ionic surfactants: [8]

The non-ionic surfactants contain bilayers lattices where the polar head and non-polar tail. To attain thermodynamic stability, every bilayers folds over itself as continuous membrane i.e. forms vesicles so that hydrocarbon /water interface remains no more exposed. [9, 10]

Mainly following types of non-ionic surfactant are used for the formation of Niosomes: - [11]

a) Alkyl ethers:[14]
b) Alkyl esters:[12]
c) Ether linked surfactant:
d) Di-alkyl chain surfactant:[13,14]

Cholesterol: Cholesterol which is a waxy steroidal metabolite is typically added to the surfactants that are nonionic in nature and provides rigidity. Cholesterol is amphiphilic in nature; therefore it aligns its OH group in the direction of aliphatic chain and aqueous phase towards hydrocarbon chain of the surfactant. [11, 15]

Nasal drug delivery to brain

Nasal route has been explored for decades for systemic delivery of drugs; these can't be given via oral route but now IN route has gained attraction and potential for direct delivery of drug from brain to the blood circulation, thus reducing the systemic exposure and hepatic/renal clearance.[3,19,20,]

Mechanism of nasal drug delivery to brain [21]:

a) Transcellular transport:[24,25] Receptor mediated endocytosis is the transport pathway of molecule through different BBB endogenous receptors.
b) Paracellular transport: In nasal epithelium, cells are connected with each other through different junctions such as tight junction, zonula adherence and macular adherence.[26]

Advantages of Niosomes: [27, 28]

c) 1. The Niosome is increase the oral bioavailability drugs which are poorly absorbed and improve drugs to penetrate skin.
d) 2. The vesicles can be made to act as a depot, where in which a controlled release of the drug is possible.
e) 3. They can be used in targeted drug delivery action by oral, parentral as well as topical routes.
f) 4. This nano Niosomes drug delivery carriers are osmotically active and it also shows greater stability.
g) 5. No particular condition is necessary for working with and storing of Niosomal formulations.
h) 6. The use of Niosomal vesicle system in cosmetics and other therapeutic activities may show various advantages.

Disadvantages of Niosomes [29]:

a) Aggregation
b) Physical instability,
c) Time consuming,
d) Leaking of Entrapped drug[30]

Materials and methods:

Artemether was supplied by IPCA Private Ltd. (Gujrat, India). Span 60 was supplied by Research lab fine chemicals Industries Mumbai. HPLC grade Methanol, Dichloromethane, Lactose and Cholesterol was purchased from Research lab fine chemicals Industries Mumbai (India). Chloroform was purchased from LOBA chemie. Pvt. Ltd., Mumbai and Neusilin carrier grade US2 and USF2 was purchased from Fuji chemical Industries, Gangawal chemicals, Mumbai .Water used was purified by reverse osmosis (MilliQ. Millipore, USA). All chemicals were analytical grade and used as received.

Preparation of Proniosomes: [16, 17, 18]

The Proniosome powder was prepared by using Slurry method [45]. The screening of the ratio by using different carrier is represented in Table No.1. In that accurately weighed amounts of Span60 and Cholesterol at various ratios (1:1, 1.5:1, 1:1.5) [46] and drug (15mg) were dissolved in 20ml solvent containing Chloroform and methanol (2:1). [41, 42] The resultant solution was transferred in 250ml RBF and add Neusilin (US2) 250mg (1 gm of carrier per 1mM of lipid mixture) in with processing in slurry [47, 48]. The flask attached to Rota evaporator (EVATOR Watts: 1200, 230 VAC, 50Hz, Sr.No:EV11. JIB.100) and solvent was evaporated under pressure at temp. 45±2°C. After solvent evaporation the product was dried at overnight in a vacuum oven at room temp. To obtain dry free flowing powders.

This procedure is repeated were from RBF attached to Rota evaporator but the Neusilin (UFL2) and next procedure Surface modified Lactose was used to prepare the Proniosome powder. This dried product was passed through sieve 60# to obtain free flowing powder and store at tightly closed containers at 4°C for further evaluations. [51, 52]

Procedure for Surface modified Lactose: [49, 50]

Surface modified Lactose was prepared using a process reported by Singh et. al. with slight modification. The inhalation grade α- lactose monohydrate was sieved to obtain particles in the range 63-90µm. Glyceryl monosterate (GMS), PEG 6000 (PEG), magnesium stearate (mg.st.) And Soya lecithin (LN) were used as force control agents to modify the surface of lactose. These force control agents were dispersed in a mixture of isopropyl alcohol, acetone, Water, (75:15:10). To this dispersion (30ml) , Lactose sample (100g) were mixed and rotated(200rev/min) and dried under vacuum for 12min at 50°C by Rota evaporator sample were reprocessed 3 times with equivalent volumes (30ml) of solvent and dried and finally sieved to obtain particles in range 63-90µM.

Composition of formulation:

Table No. 1: Composition of formulation

Formulation code	**Span 60: Cholesterol Molar ratio**	**Span60 (mg)**	**Cholesterol (mg)**	**Chloroform (ml)**	**Methanol (ml)**
F1	1:1	53.8	48.2	12.5	7.5
F2	1.5:1	64.5	38.6	12.5	7.5
F3	1:1.5	43.0	57.9	12.5	7.5

Table No. 2: Batches with processing carrier: [52]

Ratio	Batches	Drug (gm)	Span 60 (gm)	cholesterol (gm)	Solvent (%)	NUS2 (gm)	NUFL2 (gm)	SM LACTOSE (gm)
1:1NUS2	F4	15	0.584	0.960	2:1	1.88	-	-
1:1NUFL2	F5	15	0.584	0.960	2:1	-	1.88	-
1:1LACTOSE	F6	15	0.584	0.960	2:1	-	-	1.88
1.5:1NUS2	F7	15	0.645	0.386	2:1	1.88	-	-
1.5:1NUFL2	F8	15	0.645	0.386	2:1	-	1.88	-
1.5:1LACTOSE	F9	15	0.645	0.386	2:1	-	-	1.88
1:1.5NUS2	F10	15	0.430	0.579	2:1	1.88	-	-
1:1.5NUFL2	F11	15	0.430	0.579	2:1	-	1.88	-
1:1.5LACTOSE	F12	15	0.430	0.579	2:1	-	-	1.88

Ratio	Batches	Drug (gm)	Span 60 (gm)	cholesterol (gm)	Solvent (%)	NUS2 (gm)	NUFL2 (gm)	SM LACTOSE (gm)
1:1NUS2	F13	15	0.584	0.960	2:1	1.88	-	-
1:1NUFL2	F14	15	0.584	0.960	2:1	-	1.88	-
1:1LACTOSE	F15	15	0.584	0.960	2:1	-	-	1.88
1.5:1NUS2	F16	15	0.645	0.386	2:1	1.88	-	-
1.5:1NUFL2	F17	15	0.645	0.386	2:1	-	1.88	-
1.5:1LACTOSE	F18	15	0.645	0.386	2:1	-	-	1.88
1:1.5NUS2	F19	15	0.430	0.579	2:1	1.88	-	-
1:1.5NUFL2	F20	15	0.430	0.579	2:1	-	1.88	-
1:1.5LACTOSE	F21	15	0.430	0.579	2:1	-	-	1.88

Experimental work:

Calibration Curve with dichloromethane and Phosphate buffer (pH6.8): [31, 32]

a. Preparation of stock solution in Phosphate buffer (pH 6.8):
b. Determination of λ_{max} of Artemether with Phosphate buffer (pH6.8):
c. Preparation of calibration curve of Artemether in Phosphate buffer (pH6.8):
d. Preparation of stock solution in Dichloromethane:
e. Determination of λ_{max} of Artemether in Dichloromethane:
f. Preparation of calibration curve of Artemether in Dichloromethane:

5 Fourier transform Infra-red Spectroscopy (FTIR) [33, 34, 35]

EVALUATION OF PRONIOSOME POWDER: [53, 54]

Physical appearance [39]

All the prepared floating microspheres formulations of Artemether were checked for their size, shape and colour.

Micromeritic properties [40]

All the prepared Proniosome powder was checked for the bulk density, Tapped density, Carr's index, Hausner's ratio, and Angle of Repose.

a) Bulk density:
b) Tapped density:
c) Hausner's ratio:
d) Carr's index:
e) Angle of Repose:

Percentage yield [55]

Determination of drug content and drug entrapment efficiency [56, 57]

The drug content was assayed using UV-spectrophotometer (V – 630, Shimadzu Co Ltd., Japan) at 228 nm after suitable dilution with Dichloromethane.

Surface characterization [58]

Surface characterization of Proniosome powder was examined with a Scanning Electron Microscopy (SEM Sophisticated Test and Instrumentation centre, Pune).

In- vitro drug release study [59, 60]

The release of Artemether from Proniosome powder was determined by using dialysis membrane at 200rpm. The dissolution medium used 50 ml of Phosphate buffer (pH6.8) and temperature was maintained at 37^0c. A sample (5ml) was withdrawn at 30 min., 60min, 90min, 120min, 150min, 180min, 210min, and 240min. The samples were filtered through whatman filter paper and analysed using UV method. Cumulative % drug release was calculated and observed. The dissolution of the formulation was compared with the Standard Artemether capsule.

RESULT AND DISCUSSION

Organoleptic properties: [31]

Table No. 3: Organoleptic properties of Artemether

Sr.No	**Properties**	**Observation**
1	Appearance	Amorphous powder
2	Colour	white
3	Odour	Odourless

Melting point determination:

Table No.4: Melting point of Artemether

Sr. No.	**Drug**	**Melting point**	
1	Artemether	Literature	Observed
		86^0C- 90^0C(31)	88^0C

Solubility [32]

Table No. 5: Solubility determination of Artemether

Drug	Solvent	Concentration (µg/ml)
Artemether	Methanol	0.650
	Dichloromethane	0.066
	Acetone	0.099
	Water	Insoluble
	Phosphate buffer (pH 6.8)	0.170
	Chloroform	0.240

Ultraviolet –Visible spectroscopy study [33, 34, 35]

Determination of λ_{max} of Artemether

Table No. 6: Maximum wavelength of Artemether in Phosphate buffer (pH6.8)

Solvent	Wavelength of maxima (nm)
Phosphate Buffer (pH6.8)	254

Calibration curve of Artemether in Phosphate buffer (pH6.8)

Table No. 7: Absorbance value of Artemether in Phosphate buffer (pH6.8)

Sr. No.	Concentration (µg/ml)	Absorbance at 254 nm
1.	10	0.640
2	20	0.741
3.	30	0.885
4.	40	0.959
5.	50	1.110

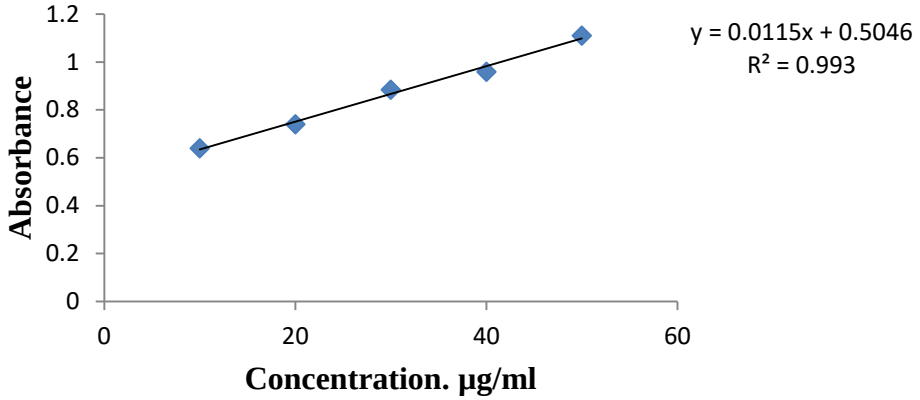

Figure 1 : Calibration curve of Artemether in Phosphate buffer (pH6.8)

Determination of λ_{max} of Artemether in Dichloromethane:

Table No. 8: Maximum wavelength of Artemether in Dichloromethane:

Solvent	**Wavelength of maxima (nm)**
Dichloromethane	228

C) Calibration curve of Artemether in Dichloromethane:

Table No. 9: Absorbance value of Artemether in Dichloromethane

Sr. No.	**Concentration (µg/ml)**	**Absorbance at 228 nm**
1.	10	0.145
2	20	0.226
3.	30	0.367
4.	40	0.425
5.	50	0.572

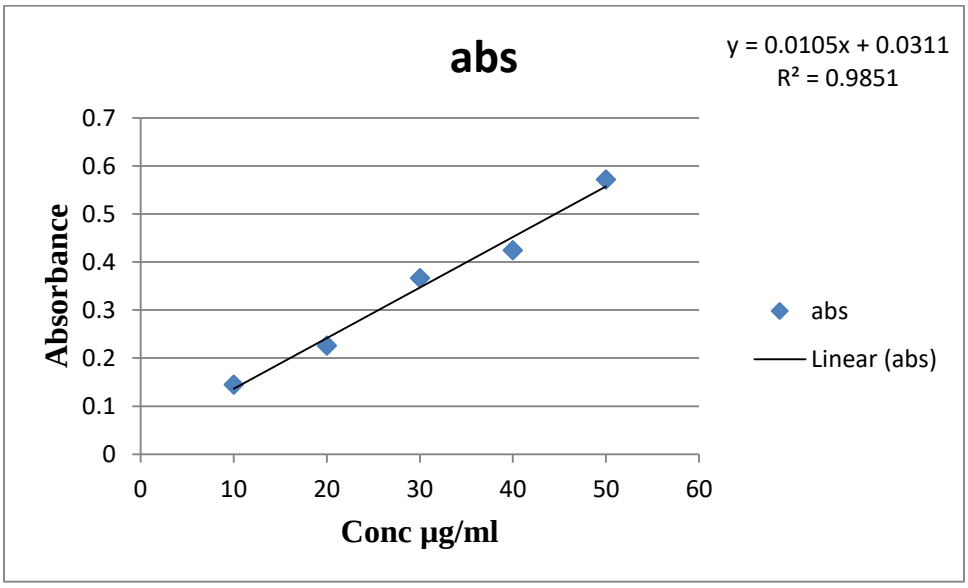

Figure 2: Calibration curve of Artemether in Dichloromethane

8.1.5 Fourier Transform Infra- Red Spectroscopy (FTIR): [36, 37,]

Infrared spectrum of Artemether is shown in fig.7. The major peaks observed and corresponding functional groups are given in Table 13. Infra-red spectrum shows peak characteristics of structure of Artemether.

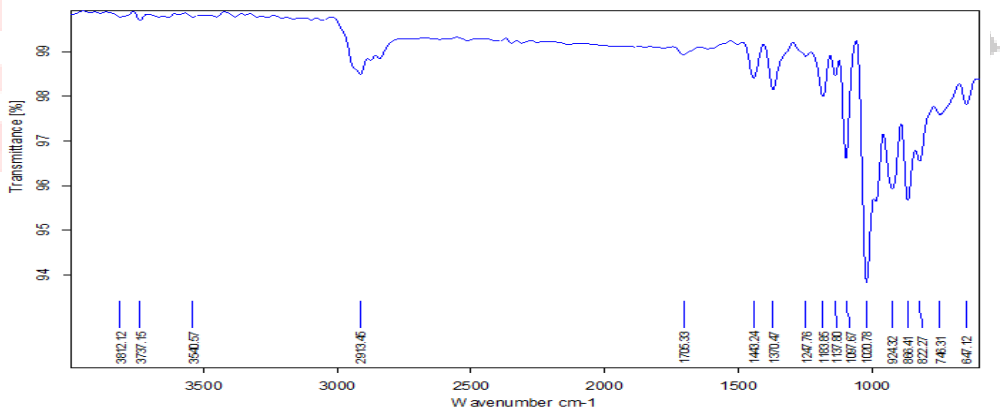

Figure 3: FTIR Spectrum of Artemether

Table No. 10: Interpretation of FTIR Spectrum of Artemether

Sr. No.	Observed values of peaks (cm^{-1})	Standard Values of peaks (cm^{-1})	Functional Group
1.	746.31	750±20	C-H Bend
2.	822.278	840-790	C=C Bend
3.	866.41	880±20	C-H Bend
4.	924.32	900-700	=C-H Bend
5.	1020.78	1075-1020	-C-F-Stretch
6.	1097.67	1020-1250	-C-F-Stretch
7.	1137.80	1020-1250	-C-F-Stretch
8.	1183.86	1020-1250	-C-F-Stretch
9.	1247.76	1020-1250	-C-F-Stretch
10.	1370.47	1370-1335	-S=O-Stretch
11.	1443.24	1450	-C-H-Stretch
12.	2913.46	3000-2840	-C-H-Stretch
13.	3540.57	3550-3200	-O-H-Stretch

8.3.2 Drug-Excipients Compatibility Study: [38, 39, 40]

Physical and chemical Compatibility study was carried out both in presence and absence of moisture at 45º C in stability chamber for 14 days. The drug-excipients mixtures were observed for physical incompatibilities such as colour change, liquefaction, caking, and gas formation and chemical incompatibilities with the help of FT-IR study. The results obtained at each day in presence and absences of moisture were given in following table 11.

Table No. 11: Compatibility study of drug and excipients mixture.

Drug + Excipients	Span60	Cholesterol	Neusilin NUS2	Neusilin NUFL2	SM Lactose
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	-	-	-	-	-
10	-	-	-	-	-
11	-	-	-	-	-
12	-	-	-	-	-
13	-	-	-	-	-
14	-	-	-	-	-

No change (-); caking (#); liquefaction- (*); gas formation- (¥)

8.4. Evaluation of Proniosomes

8.4.1 Physical Appearance

To developed formulation dissolve all the pre-requisite to become a Proniosomes.

Figure 4: Physical appearance of the formulated Proniosomes

8.4.2 Micromeritic properties The Micromeritic property (Bulk density, Tapped density, Carr's index, Hausner's ratio and angle of repose) of all the formulated batches was measured.

Table No. 12: Micromeritic properties of the formulation

Batch code	Bulk density (gm/ml) ± SD	Tapped density (gm/ml) ± SD	Carr's index ±SD	Hausner's ratio ± SD	Angle of Repose (º)
F1	0.4953 ±0.003	0.4283 ± 0.0052	7.70 ± 0.0578	1.0834± 0.0032	31.9º
F2	-				
F3	-				
F4	0.3734 ±0.006	0.349 ± 0.0357	0.5036± 0.007	1.0637± 0.0041	32.02º
F5	0.4462 ±0.005	0.322 ± 0.044	0.4862± 0.006	1.0270± 0.004	33.82º
F6	0.441 ±0.045	0.356 ±0.030	5.320 ± 0.037	1.050 ±0.021	35º
F7	0.371 ±0.104	0.497 ±0.033	8.149 ± 0.046	1.080± 0.023	36.92º
F8	0.431 ±0.060	0.434 ±0.037	8.257 ± 0.029	1.60± 0.016	36.78º
F9	-				
F10	0.300 ± 0.026	0.383 ±0.066	8.378 ±0.024	1.090 ± 0.024	35.86º
F11	0.463± 0.037	0.497 ±0.077	6.421 ±0.022	1.026 ± 0.068	39.48º
F12					
F13	0.358±0.050	0.491 ± 0.031	5.534 ± 0.031	1.046 ± 0.019	34.02º
F14	0.483 ±0.066	0.400 ± 0.026	6.490 ± 0.024	1.078 ±0.024	32.82º
F15	0.397 ±0.077	0.463± 0.037	8.726 ± 0.068	1.021 ±0.022	33º
F16	0.473 ±0.070	0.477 ± 0.042	7.823 ± 0.048	1.042 ±0.018	32.92º
F17	0.341 ±0.045	0.356 ±0.030	7.920 ± 0.037	1.050 ±0.021	34.78º
F18	-				
F19	0.377 ± 0.042	0.373 ±0.070	5.342 ±0.018	1.023 ± 0.048	32.86º
F20	0.456 ±0.030	0.541 ±0.045	6.250 ±0.021	1.020 ± 0.037	36.48º
F21	-				
Placebo	0.3731 ±0.060	0.340 ±0.037	5.157 ± 0.029	1.060± 0.016	30.9º

From the study of the Micromeritic properties of the formulation it was found that the bulk density of the formulation lies within range of 0.3614 – 0.4734 g/cm^3, tapped density within range of 0.3849-0.5036. The Carr's index lies within range of 5.99 – 8.22 and Hausner's ratio within range of 1.0270 – 1.0834 which indicates that the prepared formulation have excellent flow property.

8.4.3 Percentage yield

The percentage yield of Proniosomes of Artemether was measured.

All formulations F1 – F21 found percentage yield 97.79 – 99.26% which lied in the normal range in table no.13.

8.4.4 Drug content and drug entrapment efficiency

The Proniosomes were dissolved in Phosphate buffer pH 6.8 under sonication and filtered. The drug content was assayed using UV- spectrophotometer (V-630, Shimadzu Co Ltd., Japan) at 228nm after suitable dilution with Phosphate buffer ph6.8 Percentage drug content and percentage entrapment efficiency was determined using formula :

Table No. 13: Percentage yield of the formulations, Drug content and drug entrapment efficiency,

Batch code	Percentage yield (%)	% DEE± SD	Drug Contents(%)±SD
F1	99.26	92.83 ±0.066	97.51±0.018
F2	96.15	90.97 ±0.077	90.64±0.021
F3	97.02	90.73 ±0.070	91.33±0.023
F4	97.85	90.41 ±0.045	90.15±0.016
F5	97.08	92.71 ±0.104	99.85±0.024
F6	97.79	90.31 ±0.060	98.46±0.068
F7	98.05	91.78 ±0.024	98.93±0.048
F8	98.15	90.21 ±0.022	97.62±0.037
F9	97.20	91.42 ±0.018	95.26±0.046
F10	98.65	90.50 ±0.021	98.08±0.029
F11	97.35	90.50± 0.023	92.39±0.042
F12	98.28	91.60± 0.016	95.56±0.030
F13	97.14	92.90 ± 0.024	95.26±0.033
F14	97.98	90.26 ± 0.068	95.21±0.066
F15	97.02	91.23 ± 0.048	94.46±0.077
F16	98.45	90.20 ± 0.037	95.85±0.070
F17	97.97	90.49 ± 0.046	92.57±0.045
F18	97.06	91.57 ± 0.029	91.47±0.104
F19	98.08	90.77 ± 0.042	94.20±0.060
F20	97.32.	91.56 ±0.030	91.12±0.104
F21	97.66	90.97 ±0.033	97.19±0.060
Placebo	98.27	-	-

All formulations F1 – F21 found percentage yield 97.79 – 99.26% which lied in the normal range in table no.22. The percentage drug content of all prepared formulations was found to be in the range of 92.56 – 98.77%. Therefore uniformity of drug content was maintained in all formulations. The percentage drug entrapment efficiency of all prepared formulations was found to be in the range of 90.28% - 92.62%.

Surface characterization

Surface characterization of the Proniosomes were examined with scanning electron microscopy (Sophisticated test and instrumentation centre, Pune).Proniosomes were mounted on metal rids using double sided tape and coated with gold under vacuum.

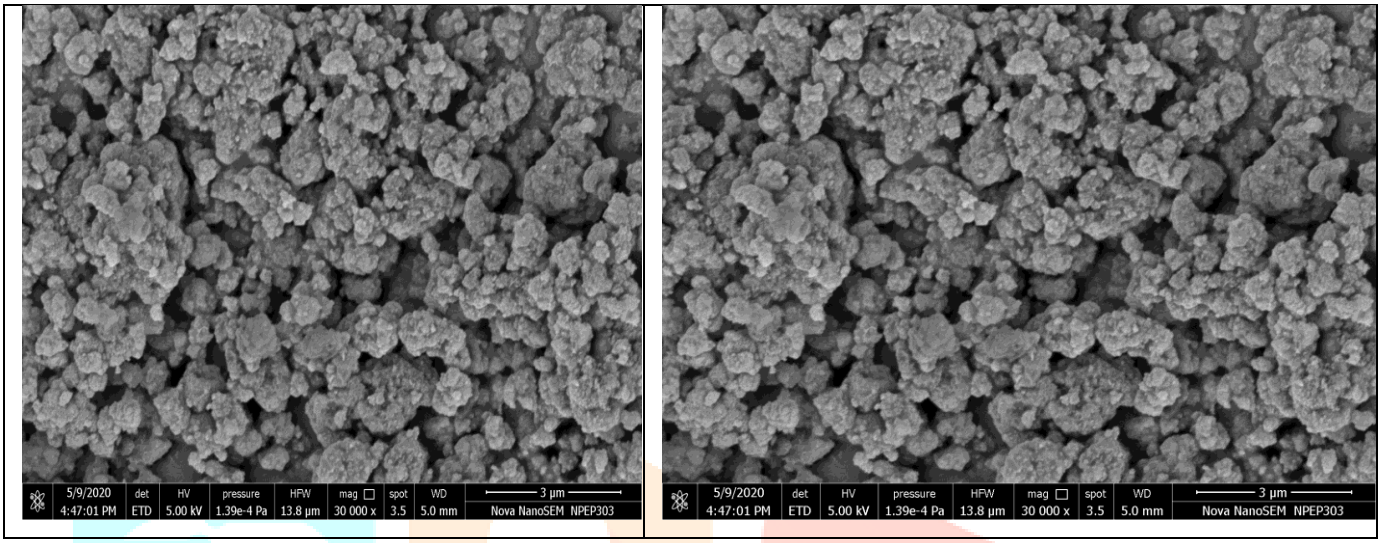

Fig. No. 5: Surface morphology of the formulation

The SEM result showed that the particle size of formulation was found to have regular and spherical shape with rough and uneven surface.

8.4.6 In vitro drug release study

The In vitro drug release study of different formulation

Table No. 14: In-vitro drug release of different batches of the formulation

Time (Min.)	**F1**	**F2**	**F3**	**Placebo**
0	**0**	**0**	**0**	**0**
30	26.58±0.050	15.34 ± 0.031	14.91 ± 0.031	10.46 ± 0.019
60	28.83 ±0.066	26.90 ± 0.024	39.00 ± 0.026	19.78 ±0.024
90	58.97 ±0.077	48.26 ± 0.068	44.63± 0.037	39.21 ±0.022
120	68.73 ±0.070	67.23 ± 0.048	61.77 ± 0.042	54.42 ±0.018
150	83.41 ±0.045	79.20 ± 0.037	73.56 ±0.030	66.50 ±0.021
180	92.71 ±0.104	80.49 ± 0.046	80.97 ±0.033	83.50± 0.023
210	97.31 ±0.060	85.57 ± 0.029	84.34 ±0.037	94.60± 0.016
Time (Min.)	**F4**	**F5**	**F6**	**F7**
0	**0**	**0**	**0**	**0**
30	16.77 ± 0.042	24.42 ±0.018	23.73 ±0.070	27.23 ± 0.048
60	21.56 ±0.030	56.50 ±0.021	28.41 ±0.045	29.20 ± 0.037
90	30.97 ±0.033	60.50± 0.023	32.71 ±0.104	40.49 ± 0.046
120	45.12 ±0.037	64.60± 0.016	53.31 ±0.060	45.57 ± 0.029
150	68.91 ± 0.031	70.46 ± 0.019	60.58±0.050	55.34 ± 0.031
180	75.00 ± 0.026	87.78 ±0.024	72.83 ±0.066	60.90 ± 0.024
210	80.63± 0.037	91.21 ±0.022	80.97 ±0.077	78.26 ± 0.068

Time (Min.)	F8	F9	F10	F11
0	**0**	**0**	**0**	**0**
30	26.58±0.050	25.34 ± 0.031	26.58±0.050	25.34 ± 0.031
60	32.83 ±0.066	30.90 ± 0.024	31.83 ±0.066	35.90 ± 0.024
90	40.97 ±0.077	48.26 ± 0.068	40.97 ±0.077	48.26 ± 0.068
120	42.71 ±0.104	52.49 ± 0.046	57.97 ±0.033	53.50± 0.023
150	46.58±0.050	65.34 ± 0.031	68.91 ± 0.031	60.46 ± 0.019
180	52.83 ±0.066	70.90 ± 0.024	75.00 ± 0.026	16.78 ±0.024
210	60.97 ±0.077	78.26 ± 0.068	84.63± 0.037	79.21 ±0.022

Time (Min.)	F12	F13	F14	F15
0	**0**	**0**	**0**	**0**
30	23.73 ±0.070	27.23 ± 0.048	21.77 ± 0.042	24.42 ±0.018
60	28.41 ±0.045	29.20 ± 0.037	33.56 ±0.030	36.50 ±0.021
90	32.71 ±0.104	40.49 ± 0.046	47.97 ±0.033	43.50± 0.023
120	53.31 ±0.060	45.57 ± 0.029	58.12 ±0.037	54.60± 0.016
150	68.91 ± 0.031	60.46 ± 0.019	66.58±0.050	65.34 ± 0.031
180	79.00 ± 0.026	69.78 ±0.024	72.83 ±0.066	70.90 ± 0.024
210	84.63± 0.037	75.21 ±0.022	80.97 ±0.077	78.26 ± 0.068

Time (Min.)	F16	F17	F18	F19
0	**0**	**0**	**0**	**0**
30	28.91 ± 0.031	10.46 ± 0.019	16.58±0.050	15.34 ± 0.031
60	39.00 ± 0.026	19.78 ±0.024	22.83 ±0.066	21.90 ± 0.024
90	44.63± 0.037	39.21 ±0.022	30.97 ±0.077	25.26 ± 0.068
120	61.77 ± 0.042	54.42 ±0.018	43.73 ±0.070	34.23 ± 0.048
150	63.56 ±0.030	66.50 ±0.021	58.41 ±0.045	46.20 ± 0.037
180	77.97 ±0.033	73.50± 0.023	62.71 ±0.104	65.49 ± 0.046
210	80.12 ±0.037	84.60± 0.016	73.31 ±0.060	75..57 ± 0.029

Time (Min.)	F20	F21
0	**0**	**0**
30	10.46 ± 0.019	15.34 ± 0.031
60	19.78 ±0.024	25.90 ± 0.024
90	29.21 ±0.022	35.26 ± 0.068
120	34.42 ±0.018	47.23 ± 0.048
150	46.50 ±0.021	59.20 ± 0.037
180	53.50± 0.023	60.49 ± 0.046
210	64.60± 0.016	75.57 ± 0.029

Maximum drug release 91.25% was shown by F5 batch. The data also suggested that Proniosomes formulation were capable to produce linear drug release for longer period of time. Drug release profile of formulation F1 to F11 shown in Fig 6 and drug release profile F12 to F21 signified sustained drug release. Out of four formulations maximum release after 4 hr was found for F5 formulation.

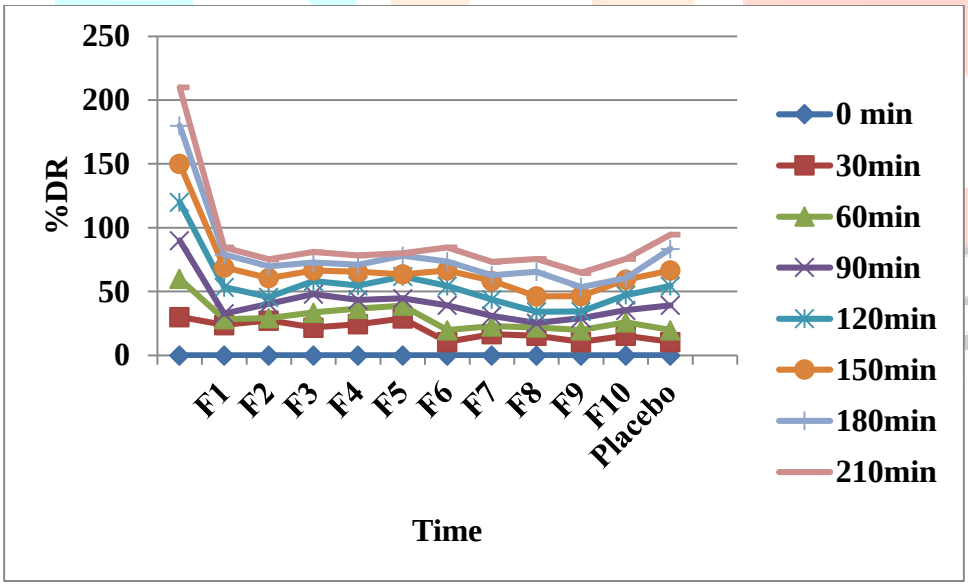

Figure 6: Drug release profile of formulations F1-F11

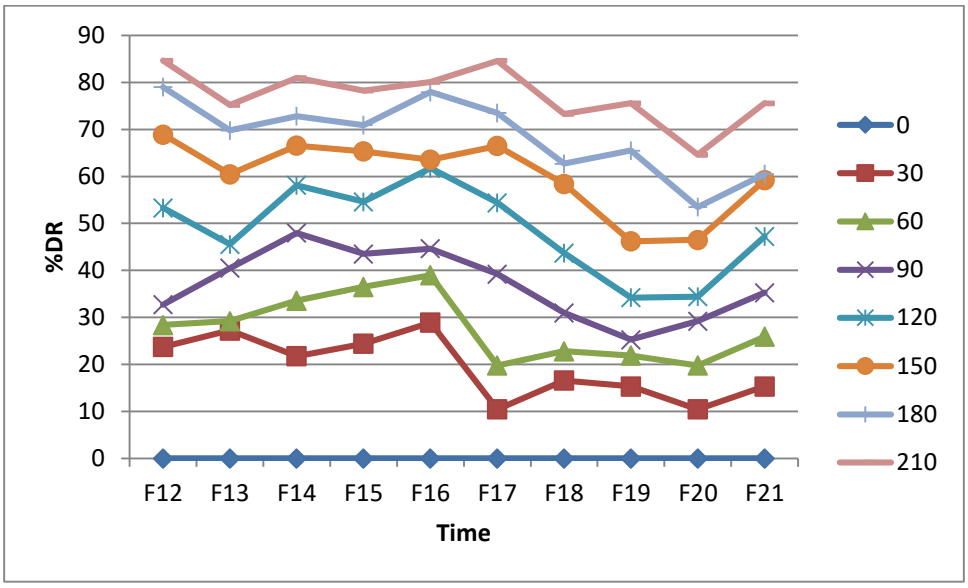

Figure7: Drug release profile of formulations F12-F21

8.4.11 Permeation study

The Permeation study of different formulation

Table No. 15: In-vitro drug release of different batches of the formulation

Time (Min.)	**F1**	**F2**	**F3**	**Placebo**
0	**0**	**0**	**0**	**0**
30	20.58±0.050	10.34 ± 0.031	8.91 ± 0.031	10.46 ± 0.019
60	35.83 ±0.066	15.90 ± 0.024	19.00 ± 0.026	19.78 ±0.024
90	47.97 ±0.077	18.26 ± 0.068	34.63± 0.037	39.21 ±0.022
120	59.73 ±0.070	27.23 ± 0.048	51.77 ± 0.042	54.42 ±0.018
150	81.41 ±0.045	39.20 ± 0.037	63.56 ±0.030	66.50 ±0.021
180	91.71 ±0.104	50.49 ± 0.046	77.97 ±0.033	83.50± 0.023
210	97.31 ±0.060	75.57 ± 0.029	80.12 ±0.037	94.60± 0.016
Time (Min.)	**F4**	**F5**	**F6**	**F7**
0	**0**	**0**	**0**	**0**
30	16.77 ± 0.042	19.42 ±0.018	23.73 ±0.070	27.23 ± 0.048
60	29.56 ±0.030	26.50 ±0.021	28.41 ±0.045	29.20 ± 0.037
90	37.97 ±0.033	39.50± 0.023	32.71 ±0.104	40.49 ± 0.046
120	48.12 ±0.037	59.60± 0.016	53.31 ±0.060	45.57 ± 0.029
150	58.91 ± 0.031	65.46 ± 0.019	66.58±0.050	55.34 ± 0.031
180	69.00 ± 0.026	86.78 ±0.024	72.83 ±0.066	70.90 ± 0.024
210	84.63± 0.037	98.21 ±0.022	80.97 ±0.077	78.26 ± 0.068

Time (Min.)	F8	F9	F10	F11
0	**0**	**0**	**0**	**0**
30	6.58±0.050	5.34 ± 0.031	16.58±0.050	15.34 ± 0.031
60	12.83 ±0.066	10.90 ± 0.024	22.83 ±0.066	25.90 ± 0.024
90	20.97 ±0.077	18.26 ± 0.068	30.97 ±0.077	38.26 ± 0.068
120	32.71 ±0.104	40.49 ± 0.046	47.97 ±0.033	43.50± 0.023
150	46.58±0.050	52.34 ± 0.031	58.91 ± 0.031	50.46 ± 0.019
180	62.83 ±0.066	60.90 ± 0.024	69.00 ± 0.026	69.78 ±0.024
210	70.97 ±0.077	78.26 ± 0.068	74.63± 0.037	79.21 ±0.022

Time (Min.)	F12	F13	F14	F15
0	**0**	**0**	**0**	**0**
30	13.73 ±0.070	17.23 ± 0.048	11.77 ± 0.042	14.42 ±0.018
60	28.41 ±0.045	29.20 ± 0.037	23.56 ±0.030	26.50 ±0.021
90	32.71 ±0.104	40.49 ± 0.046	37.97 ±0.033	33.50± 0.023
120	43.31 ±0.060	45.57 ± 0.029	48.12 ±0.037	44.60± 0.016
150	58.91 ± 0.031	50.46 ± 0.019	56.58±0.050	55.34 ± 0.031
180	69.00 ± 0.026	64.78 ±0.024	62.83 ±0.066	70.90 ± 0.024
210	84.63± 0.037	86.21 ±0.022	70.97 ±0.077	78.26 ± 0.068

Time (Min.)	F16	F17	F18	F19
0	**0**	**0**	**0**	**0**
30	18.91 ± 0.031	10.46 ± 0.019	17.58±0.050	15.34 ± 0.031
60	29.00 ± 0.026	19.78 ±0.024	28.83 ±0.066	24.90 ± 0.024
90	34.63± 0.037	39.21 ±0.022	37.97 ±0.077	38.26 ± 0.068
120	41.77 ± 0.042	47.42 ±0.018	49.73 ±0.070	47.23 ± 0.048
150	53.56 ±0.030	56.50 ±0.021	58.41 ±0.045	59.20 ± 0.037
180	67.97 ±0.033	63.50± 0.023	72.71 ±0.104	70.49 ± 0.046
210	88.12 ±0.037	74.60± 0.016	83.31 ±0.060	85.57 ± 0.029

Time (Min.)	F20	F21
0	**0**	**0**
30	14.63± 0.037	19.21 ±0.022
60	21.77 ± 0.042	29.42 ±0.018
90	33.56 ±0.030	36.50 ±0.021
120	47.97 ±0.033	43.50± 0.023
150	58.12 ±0.037	64.60± 0.016
180	62.83 ±0.066	70.90 ± 0.024
210	70.97 ±0.077	88.26 ± 0.068

Maximum permeation of drug release 98.21 % was shown by F5 batch. The data also suggested that Proniosomes formulation were capable to produce linear drug release for longer period of time. Drug release profile of formulation F1 to F11 shown in Fig 8 and dissolution profile F12 to F21 signified sustained drug release. Out of four formulations maximum release after 4 hr was found for F5 formulation.

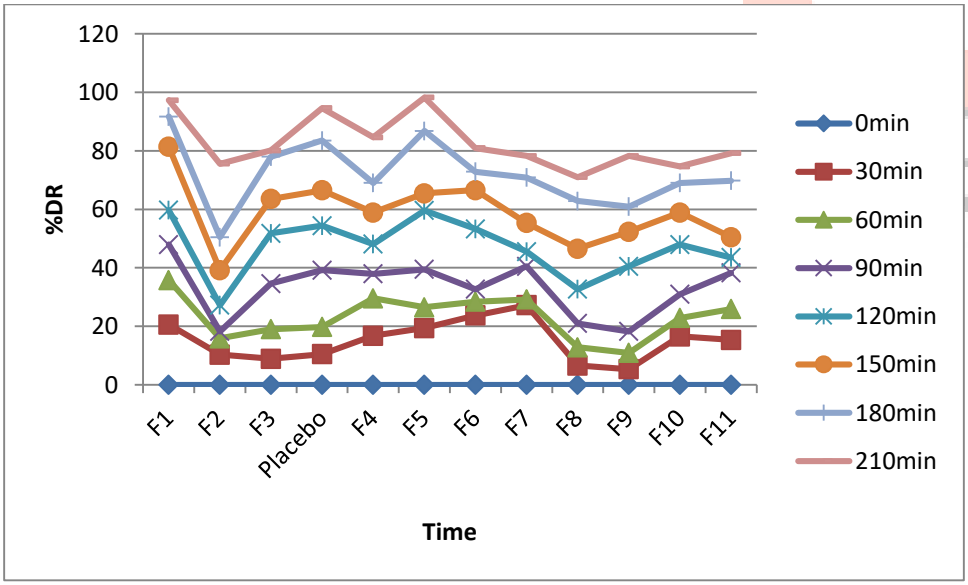

Figure 8: Drug Permeation profile of formulations F1-F11

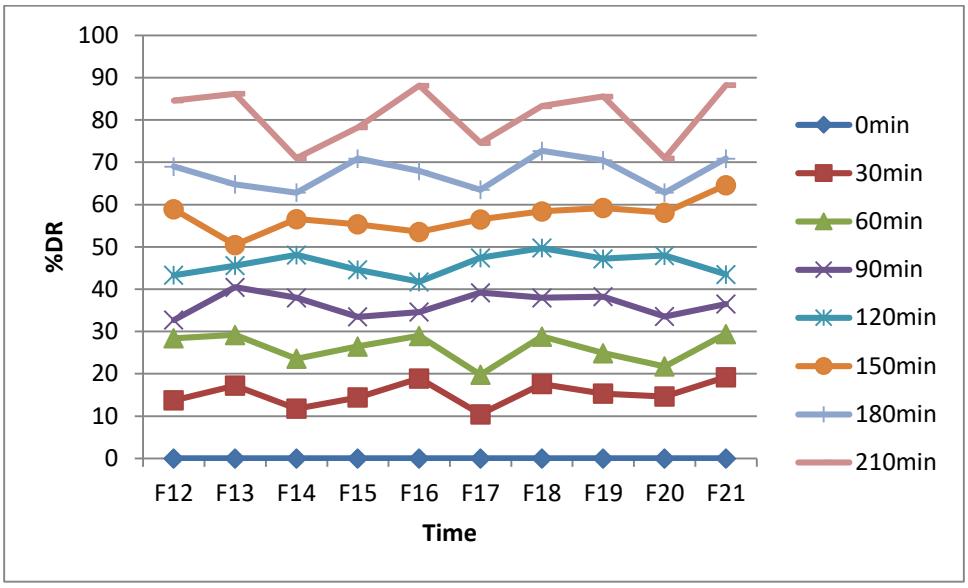

Figure 9: Drug Permeation profile of formulations F12-F21

8.6 Stability study

The sample were withdrawn after 1, 2 and 3 months and subjected to following tests a shown in Table

Table No. 16: Details of stability study for F2 batch

Test	**Before**	**After**		
	0 month	**1 month**	**2 month**	**3 month**
Drug release	94.60 ± 0.246%	94.60±0.246%	95.07±0.248	95.45±0.251
Floating lag time	>12 hrs	>12hrs	>12hrs	>12hrs

The accelerated stability studies (carried for 3 months), at temperature of 40^0C ± 2^0C and % RH 75% ± 5% RH indicated that the developed Proniosomes were unaffected after 03 months storage under accelerated condition as no change was observed in the appearance and colour of the formulation and also Taking a FTIR Spectra of Formulation are Showing no changes after 3 months. On the basis of these results, it may be concluded that the F5 formulation developed is stable under accelerated condition of 03 months.

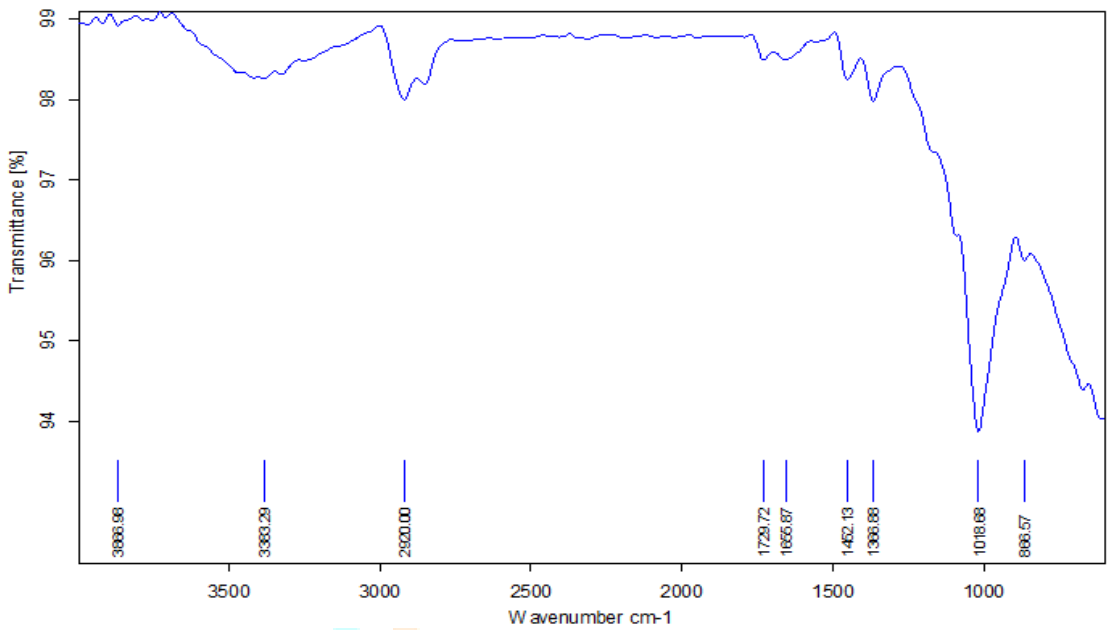

Figure 10: FTIR Spectrum of Batch F5

Table No. 17: Interpretation of FTIR spectra of Batch F5

Sr. No.	Observed values of peaks (cm^{-1})	Standard Values of peaks (cm^{-1})	Functional Group
1.	866.57	850-555	C-Cl stretch.
2.	1018.68	1075-1020	C-O stretch.
3.	1386.88	1390-1310	O-H bending.
4.	1452.13	1465	C-H bending.
5.	1666.87	1685-1666	C=O stretch.
6.	1729.72	1740-1720	C=O stretch.
7.	2900	3000-2840	C-H stretch.
8.	3333.29	3350-3310	N-H stretch.
9.	3666.98	3700-3584	O-H Stretch.

FT-IR spectra of Formulation Batch F5 after 3 months:

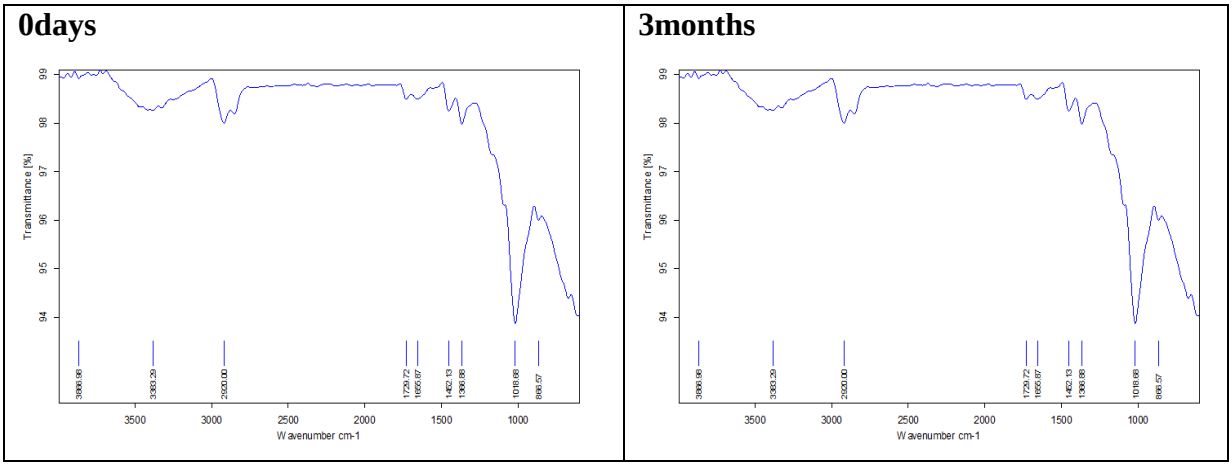

Figure 11: FTIR Spectrum of Batch F5 after 3 Months

The FTIR spectrum of Formulation Batch F5 retained all characteristics peaks visible in after 3 months.

Summary and Conclusion:

The optimized Proniosome powder was successfully developed with vesicle size small enough to facilitate the intranasal delivery of Artemether along with high encapsulation efficiency. The Artemether showed significantly improved permeation enhancement and stability with better control over drug release for a longer period through intranasal administration. Therefore, it can be concluded that Artemether-loaded Proniosome powder for intranasal delivery could be a promising platform with prolonged intranasal retention time to improve its bioavailability. It is based upon the evalutions.

The optimized Proniosome powder was gives a better result with **batch F5**. In that batch used a Neusilin NUFL2 grade gives a satisfactory result than other carriers.

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