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Biomedical Applications of Flavonoid Loaded Mesoporous Nanoparticles for the Treatment of Hepatocellular Carcinoma

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Abstract: Our research indicates that NLC is the optimal formulation for delivering anticonvulsant herbal drugs like Convoline, based on the data presented above. Future research will focus on overcoming the benefits and drawbacks of turning convoline into a dosage form and determining the precise component responsible for the anticonvulsant action of convoline. In order to file for a patent after determining the pharmacokinetic and pharmacodynamic investigations in human volunteers. With the data we selected, we were able to It was determined that Convoline, a poorly soluble and permeable herbal medicine, required an unique dosage form carrier molecule (NLC) to ensure its delivery to the bloodstream. Low soluble and low permeability herbal medications, which fall under BCS class IV, were specifically engineered to be included into a new NLC carrier. It was hypothesised that NLC nanocarriers might be used to transport a wide variety of poorly soluble and poorly permeable herbal drugs.

1. INTRODUCTION

The aim of present study was to formulate and *invitro-in vivo* evaluation of NLC and SLN drug delivery system of poorly water soluble herbal drug *Linalool*. *Linalool (coriandrol)* crude extract is extracted from *coriandrum sativum* seeds by using dichloromethane (soxhlet method) and *linalool* is isolated by using column chromatography and concentrated under reduced pressure by using rotary flash evaporator. Linalool, an essential of *Coriandrum sativum* with anti-epileptic activity. It is poorly water soluble drug; the oral delivery of *linalool* is very difficult due to variable dissolution and low bioavailability. By testing various chemical tests and TLC the confirmation of linalool was achieved. The calibration curve of linalool was prepared in dichloromethane in the concentration range of 0.04-0.2 g/ml by measuring absorbance at 665 nm. Correlation coefficient of standard curve was found to be 0.9993 in dichloromethane, indicating good linearity.The drug and excipients compatibility study was done by using BRUKER-FTIR-ATR spectrophotometer and conformed there no incompatibility between drug and excipients. Tween 80 and PEG-200 were selected as surfactant and co-surfactant respectively for the formulation of linalool SLN and NLC. Campritol was used as solid lipid for SLN and along with Campritol, Softigen was used as liquid lipid for NLC formulation.Different ratios of surfactant (tween80) and co-surfactant (PEG-200) in fixed ratios were used to prepare SLN and NLC. Drug was kept constant for all formulations and characterized for surface morphology of the NLC using scanning electron microscopy (SEM), particle size analysis, poly dispersibility index and zeta potential measurement were done using a horiba zetasizer.Invivo studies shows that the NLC N5

treated animal shows significant (P<0.5) difference in good and quick recovery time period of convulsant when compared to other treated animal groups and also NLC shows same recovery time period when compared to marketed eptoin standard administered group.

Method

Extraction of *linalool*:
Extraction by using Soxhlet apparatus-Dichloromethane extraction process:
Sample of coriander seeds (100.0 g) was extracted by methylene chloride (500 mL) using Soxhlet apparatus. After 6 cycles of the extract, the solvent was evaporated under vacuum and the obtained extract was dried under vacuum (50^{o} C, 24 hours), resulting in the liquid (oil like) extract.

Figure: 1 Soxhlet apparatus used for extraction of crude drug from Coriandrum Sativum seeds Column chromatography for isolation

About 30 gm. of the extract was loaded on top of activated silica gel which was packed into a glass column (5x54cm). The column was eluted with a solvent gradient of hexane: ethyl acetate (9:1 ratio v/v) at a flow rate of 1mL/min. The column was then washed with 100% ethyl acetate, Total of all fractions measuring about 500 mL each were collected and concentrated using rotary evaporator. Each fraction was weighed and stored at 25^{0}C.

Figure: 2 Column packing, Loading of crude extracts &Separation of compounds

Figure: 3 Isolation and Collection of individual compound separately

Figure: 4 Different compounds isolated by column chromatography

Thin Layer Chromatography (TLC)

Figure: 5 Concentration of isolated compounds by Rotary Flash Evaporator

Glass plates (10x10 cm) were coated in our laboratory using Silica gel G, thickness 0.25 mm. Essential oil samples were dissolved in methylene chloride (ratio 1 : 10) and essential oil solutions (about 20 µL) were spotted on the plate as a start point or line. The mobile phase was toluene: ethylacetate (93:7; V/V). The development was performed at room temperature (about 20^{o}C) in a glass chamber. Detection was done by spraying the plate with 1% vanillin solution (1 g of vanillin was dissolved in 99 g mixture of 95% ethanol and cc. sulphuric acid, ratio 9:1; w/w). After spraying, the plate was heated at 110^{o}C for 5-10 min.

Figure: 6 Development of TLC plate and Detection of TLC plate by vanillin which shows the presence of linalool

Identification of drug by UV-spectro photometric method:

Preparation of standard calibration curve of *Linalool (coriandrol)* in Dichloromethane
10g of isolated Linalool was accurately weighed and dissolved in 10ml of Dichloromethane. This pipette out 0.4, 0.8, 1.2, 1.6, 2.0 ml into 10ml volumetric flask and make up the volume with Dichloromethane. Ultraviolet scan was taken between the wavelengths 400-800nm against Dichloromethane as the blank which gave a highest peak at 665 nm and the same was selected as λmax of *Linalool*. The observations were recorded in fig no: 7.

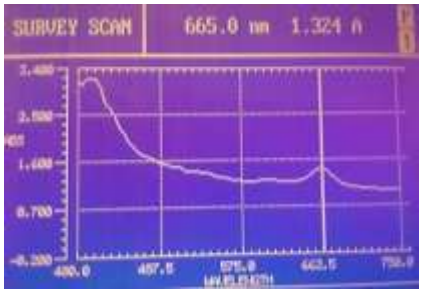


Figure: 7 Lambda max of *linalool* at 665nm

Identification of drug by IR- spectro photometric method:

Infrared spectrum of any compound or drugs gives information about the groups present in that particular compound. A spectrophotometer for recording the spectrum in the infra-red region consists of an optical system capable of providing the monochromatic light in the scanning range was 400-4000 cm-1 and resolution was 2cm-1. Attenuated total reflection (ATR) is used to analyze liquid and semi-solid samples for IR study. A drop of sample is directly place on the stage of (ATR) and scanned from 4000 cm^{-1} to 400 $cm^{-1.}$ The infrared spectrum of the drug sample was obtained using Bruker FTIR and ATR spectrophotometer (Germany) using opus software. The important peaks are reported in table and graphically represented in fig 8. this FTIR spectrum was compared with the IR of reference IR from net.

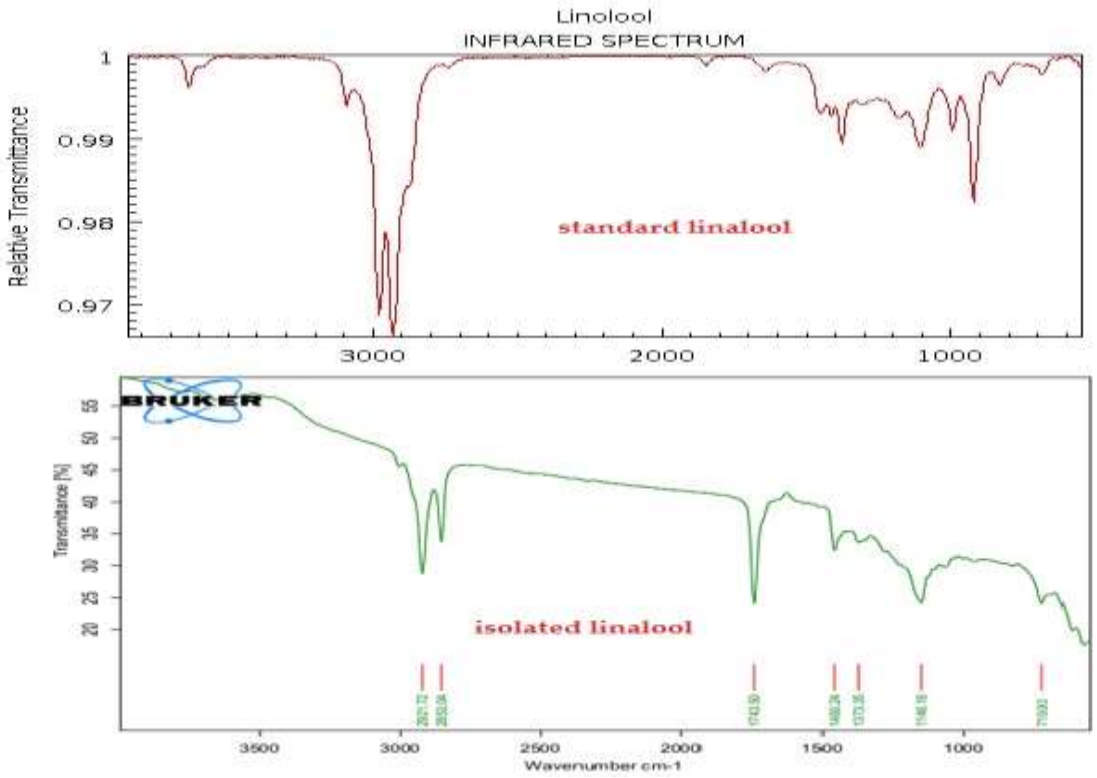


Figure: 8 Comparison on isolated linalool FTIR with standard

Preparation of calibration curve of *Linalool (coriandrol)* in Dichloromethane

10g of isolated *Linalool* was accurately weighed and dissolved in 10ml of Dichloromethane. This pipette out 0.4, 0.8, 1.2, 1.6, 2.0 ml into 10ml volumetric flask and make up the volume with Dichloromethane. Ultraviolet scan was taken between the wavelengths 400-800nm against Dichloromethane as the blank which gave a highest peak at 665 nm and the same was selected as λmax of *Linalool.*

Table No: 1 Calibration curve of linalool

S.No	Concentration g/ml	Absorbance at 665 nm
1.	0	0
2.	0.04	0.212
3.	0.08	0.416
4.	0.12	0.615
5.	0.16	0.825
6.	0.20	0.982

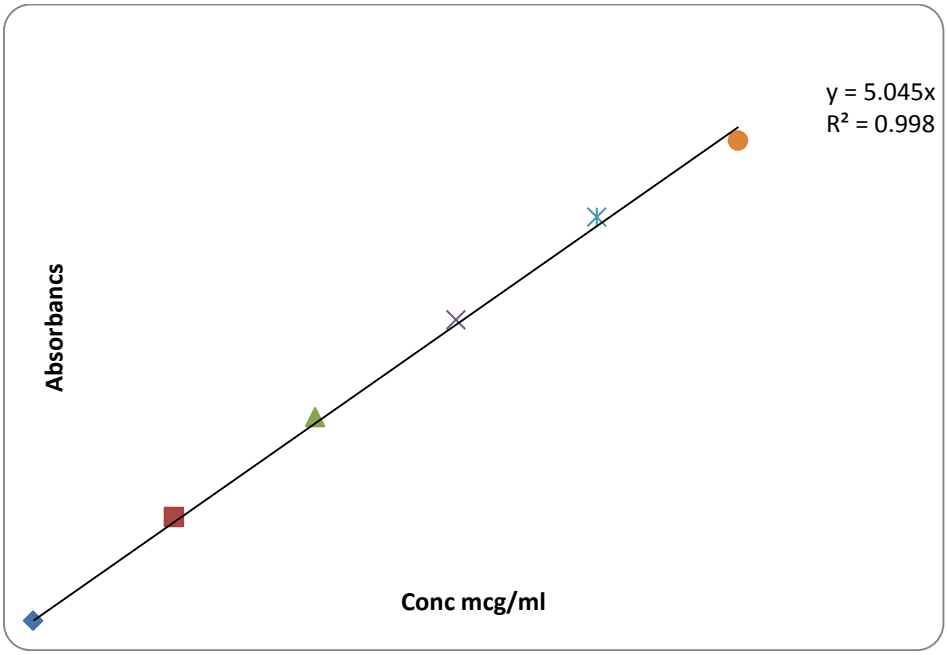


Figure: 9 Calibration curve for linalool

DRUG AND EXCIPIENT COMPATIBILITY STUDY BY FTIR:

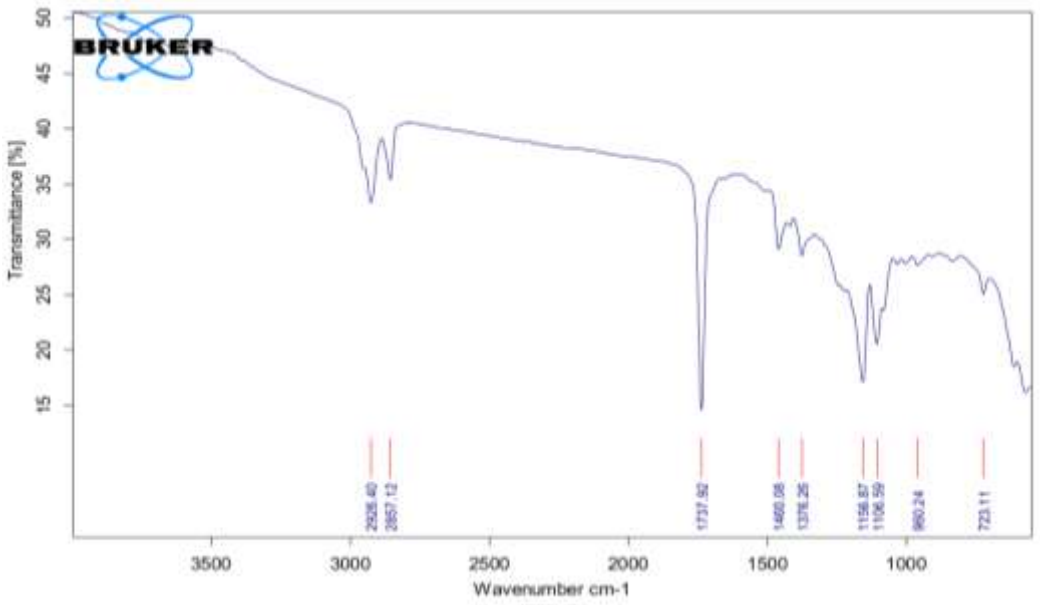


Figure: 10 FTIR spectra of Linalool + Campritol +PEG-200 (Co-surfactant) + Tween 80 (surfactant) in SLN

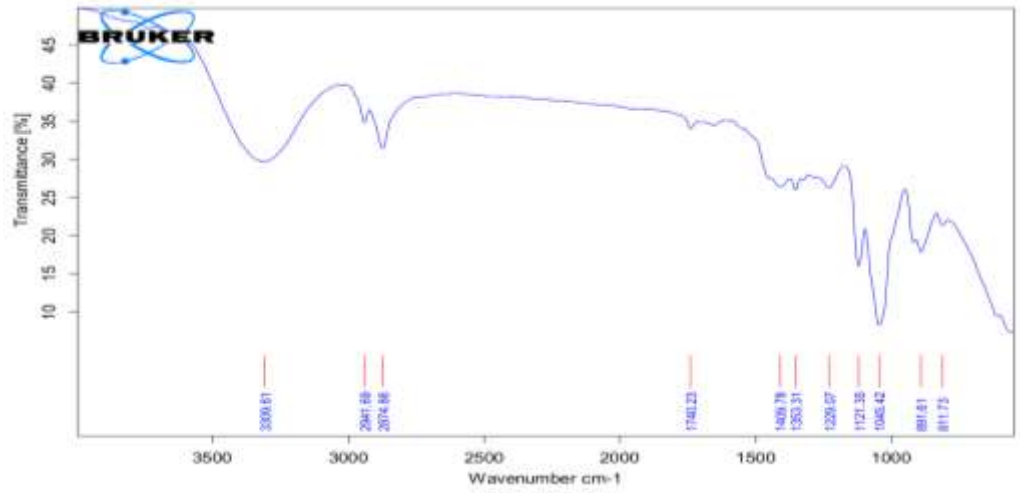


Figure: 11 FTIR spectra of Linalool + Campritol + Softigen+PEG-200 (Co-surfactant) + Tween 80 (surfactant) in NLC

The linalool SLN and NLC shown same spectra as isolated drug which demonstrates that the chemical structure of the drug, doesn't show any change after converting to SLN and NLC formulation and it confirms there is no interaction between the drug and excipients.

Preparation of SLN /NLC

Hot Homogenization Technique

The active fractional extract compound containing melted lipid is dispersed in the hot surfactant solution at the same temperature applying high-speed stirring or alternatively an

ultrasound procedure. These procedures involve the break-up of large droplets into smaller ones. The obtained pre-emulsion is passed through a high pressure homogenizer. The number of homogenization cycles ranges usually between 3 and 5, applying most of the times a pressure ranging from 500 bar to 1000-1500 bar. An aqueous dispersion of lipid nanoparticles is formed by cooling of the obtained nanoemulsion, when the lipid phase solidifies at room temperature or at temperatures below. Higher homogenization pressures are not necessary because in this case the material that is being processed is of lipid nature. The amount of energy introduced to the product should not be higher than the required to achieve the desired effects (nanometer range), as the increasing pressure will result in an increase of the costs for the energy (proportional) but also for maintenance (exponential). In many cases it might be more profitable increasing the number of homogenization cycles at a moderate pressure level rather than applying one or two passes at a higher pressure. Loaded SLN /NLC was finally obtained by allowing the hot nanoemulsion to cool to room temperature, and was stored at 4°C in the refrigerator.

Table no: 2 Composition of SLN drug delivery systems formulations of linalool

Formulation Code	Linalool (mg)	Compritol 888 ATO (solid lipid)	Tween80	PEG-200
S1	100	1	0.5	0.5
S2	100	1	1.0	0.5
S3	100	1	1.5	0.5
S4	100	1	2.0	0.5
S5	100	2	0.5	0.5
S6	100	2	1.0	0.5
S7	100	2	1.5	0.5
S8	100	2	2.0	0.5

Table no: 3 Composition of NLC drug delivery systems formulations of linalool

Formulation Code	Linalool (mg)	Compritol 888 ATO + Softigen (solid lipid + liquid lipid)	Tween80	PEG-200
N1	100	1	0.5	0.5
N2	100	1	1.0	0.5
N3	100	1	1.5	0.5
N4	100	1	2.0	0.5
N5	100	2	0.5	0.5
N6	100	2	1.0	0.5
N7	100	2	1.5	0.5
N8	100	2	2.0	0.5

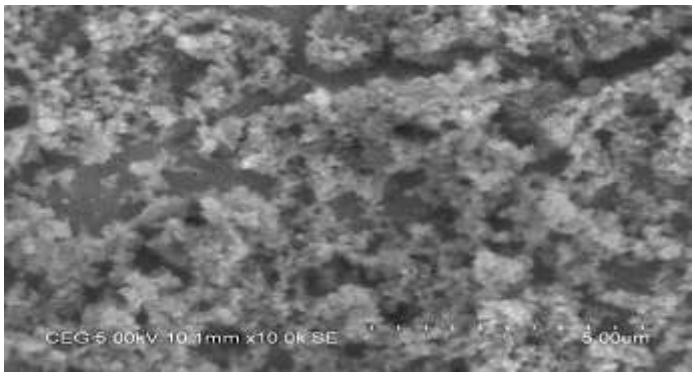
Table no: 4 Thermodynamic stability studies of SLN formulations S1-S8

Formulation	Heating Cooling Cycles (4^0C to 45^0C 72 hrs)	Freeze Thaw Cycle between -21^0C to -25^0C	Centrifugation (3500 rpm 48 hrs)	Test Result Pass/Fail
S1	Stable	Phase separation	Phase separation	Fail
S2	Stable	Phase separation	Phase separation	Fail
S3	Stable	Phase separation	Phase separation	Fail
S4	Stable	Stable	Stable	Pass
S5	Stable	Stable	Stable	Pass
S6	Stable	Stable	Stable	Pass
S7	Stable	Phase separation	Phase separation	Fail
S8	Stable	Phase separation	Phase separation	Fail

Table no: 5 Thermodynamic stability studies of NLC formulations N1-N8

Formulation	Heating Cooling Cycles (4^0C to 45^0C 72 hrs)	Freeze Thaw Cycle between -21^0C to -25^0C	Centrifugation (3500 rpm 48 hrs)	Test Result Pass/Fail
N1	Stable	Phase separation	Phase separation	Fail
N2	Stable	Phase separation	Phase separation	Fail
N3	Stable	Stable	Stable	Pass
N4	Stable	Stable	Stable	Pass
N5	Stable	Stable	Stable	Pass
N6	Stable	Phase separation	Phase separation	Fail
N7	Stable	Phase separation	Phase separation	Fail
N8	Stable	Phase separation	Phase separation	Fail

SCANNING ELECTRON MICROSCOPY (SEM):



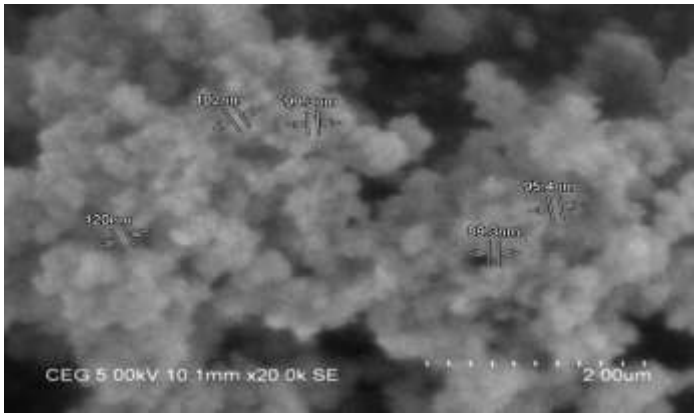


Figure: 12 SEM image of SLN S6 and SEM image of NLC N5 SLN AND NLC

PARTICLE SIZE ANALYSIS

The average particle size of SLN/ was determined by dynamic light scattering (DLS) at scattering angle 173^0C and temperature of sample holder is about 25^0C by using (Nanopartica SZ-100 HORIBA Scientific, japan). The sample of dispersion was diluted to 1:2500 v/v with double distilled water to ensure that the light scattering intensity was within the instruments range the mean particle size, poly dispersibility index and zeta potential of formulations were tabulated below Table no: 5.

Table no: 6 Particle size, polydispersicity index and zeta potential of various SLN/NLC formulations

Formulation	**Mean particle size (nm)**	**Poly dispersibility index**	**Zeta potential (mV)**
S4	231.8	0.579	-12.7
S5	205.1	0.097	-15.8
S6	**192.1**	**0.446**	**-13.7**
N3	160.7	0.618	--11.1
N4	128.1	0.771	-11.2
N5	**55.6**	**0.931**	**-31.6**
Normal range	Upto 100-500 nm	0.3 to1	±30mV

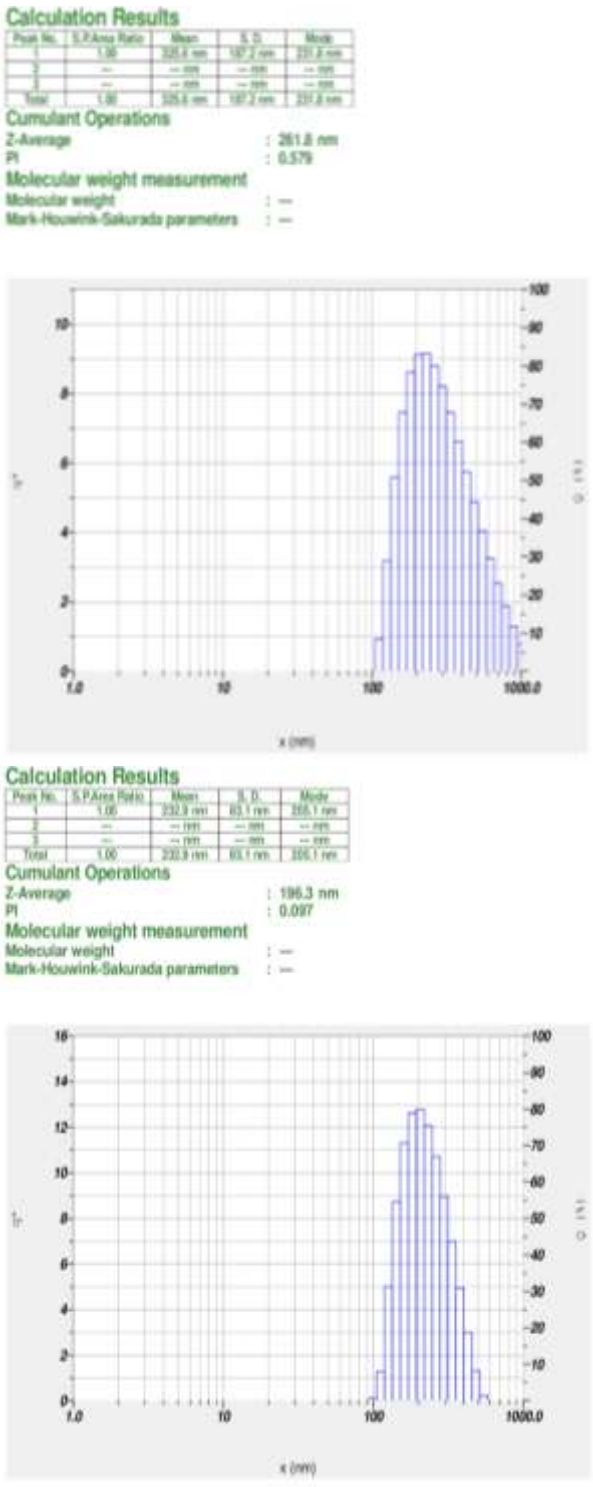


Figure: 13 Particle size distribution report for formulation SLN S4, S5 by Zeta Sizer

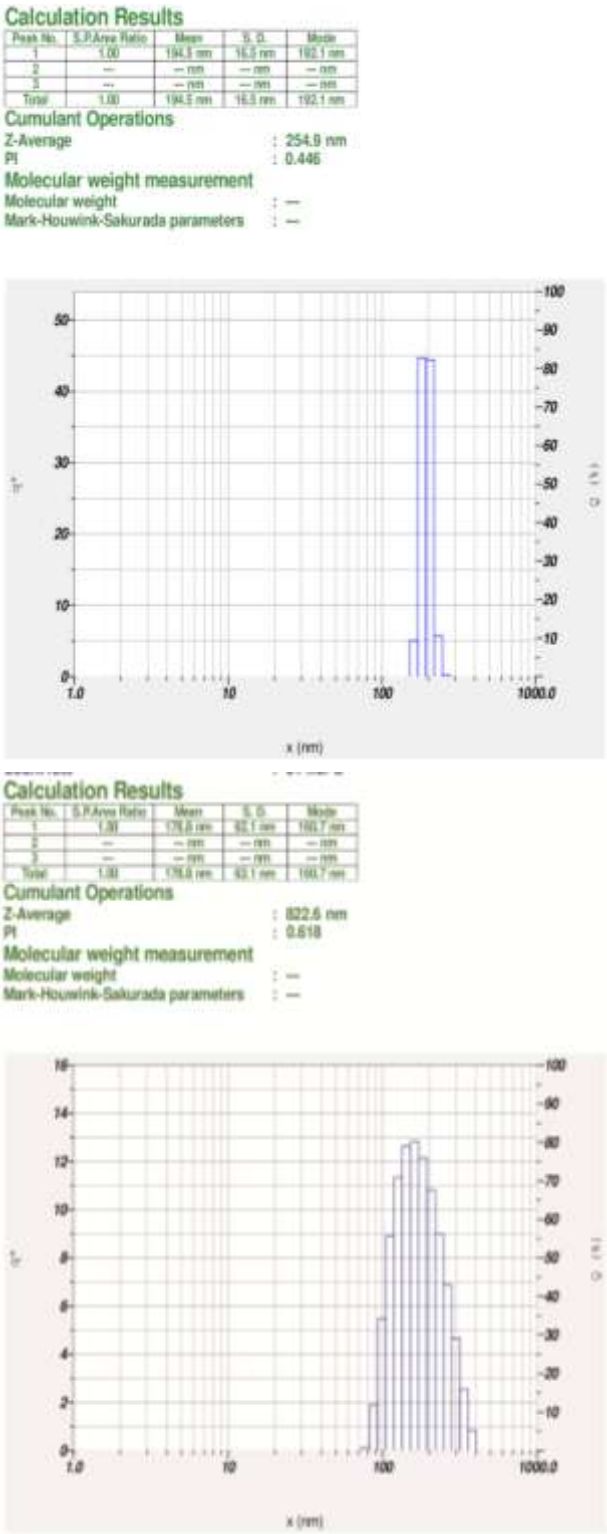


Figure: 14 Particle size distribution report for formulation SLN S6, NLC N3 by Zeta Sizer

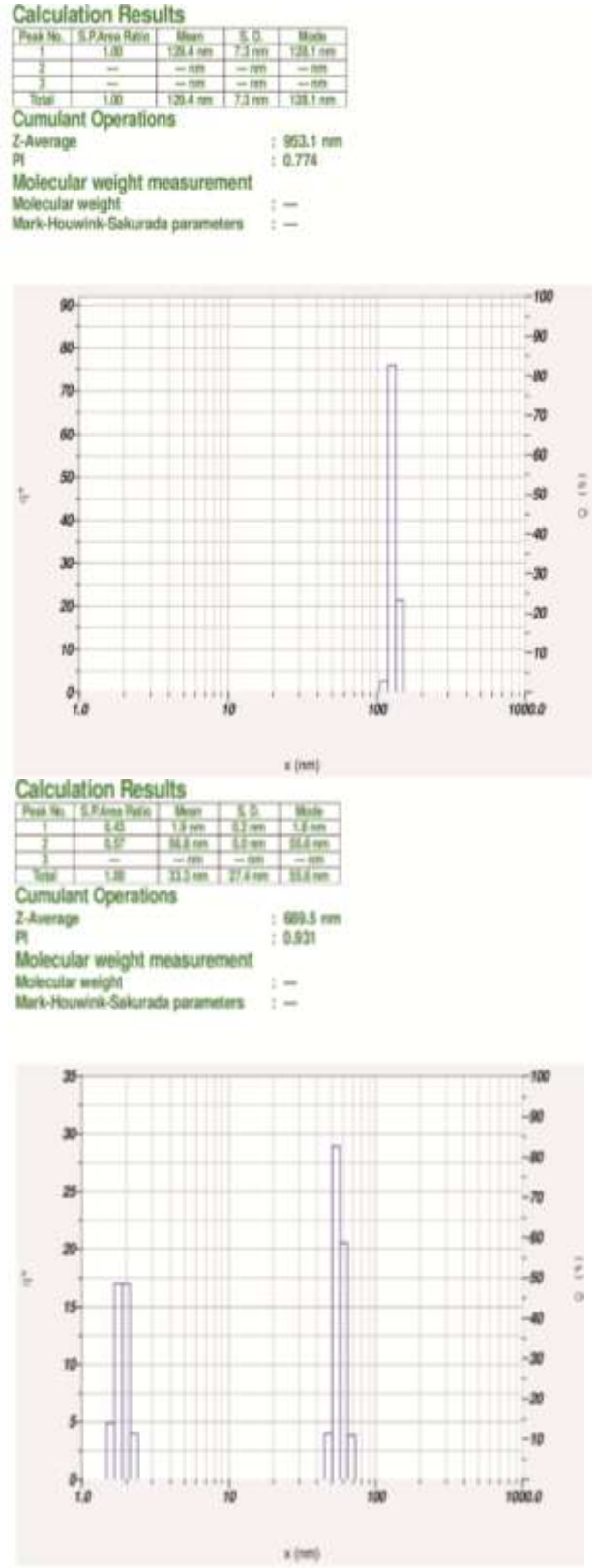


Figure: 15 Particle size distribution report for formulation NLC N4,N5 by Zeta Sizer

Discussion/Inference:
The mean Particle size of all NLC/SLN was < 250 nm shown in Figure no:13-15 the particle size was decreased with increase in Surfactant concentration in formulations. The Particle size of formulation S6 and N5 was significantly lower (192.1nm and 55.6 nm) compared with other formulations. All formulations had particles in the range which is well evident from the values of polydispersity. All SLNs/ NLCs had values of polydispersity, which indicates the uniformity of particles in the formulations. Polydispersity value was < 0.9 in the all formulations (0.579-0.931) which shows the good dispersibility of particles in the phase.

Zeta potential measurement:

Discussion/Inference:
The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. For molecules and particles that are small in size, and having high zeta potential (positive or negative) will conforms stability. When the potential is low, attraction exceeds repulsion and the dispersion will breakdown and flocculate. All the formulation of SLN S4, S5, S6 having appreciable zeta potential i.e. -12.7mV, -15.8 mV, -13.7mV, and NLC N3,N4,N5 --11.1 mV, -11.2 mV and -31.6 mV respectively. It was found that the high value of zeta potential for SLN formulation was found to be -15.8 mV for S5 formulation and -31.6 for N5 formulation. Which shows good stability of formulation?

Determination of % Drug Content
The lipid based NLC and SLN of linalool were analysed spectrophotometrically for the drug content at wave length 665 nm with proper dilution of formulations taking dichloromethane as a blank and the results are shown in table 6.All the formulation shows desired drug content.

Table No: 7 Data showing percentage drug content for SLN and NLC formulations

Formulations	% of drug content
S4	97.69±0.13
S5	97.31±0.23
S6	98.65±0.23
N3	98.27±0.65
N4	99.04±0.52
N5	99.23±0.16

IN-VITRO DRUG RELEASE STUDY:
The percentage (%) in-vitro drug release from formulations was used to measure the consistency and sustainability of drug release from the formution. The USP06 station, dissolution apparatus used to study the drug release from NLC and SLN. Dialysis membrane filled SLN and NLC was taken in the basket type dissolution apparatus in dissolution media. 900 ml of phosphate buffer pH (6.8) was used as dissolution media. The bath temperature as well as bowl Temperature was maintained about 37 ± 0.5^0C and paddle allowed rotating 75 rpm. 1ml of sample was withdrawn at different time intervals up to 12 hrs and dilution was made to 5ml. 1ml of fresh medium was replaced. The diluted samples are analysed

spectrophotometrically at 665nm and % drug release was calculated. The percentage drug release of S6 and N5 formulation shows better control of drug release.S6 shows 88.54 ± 2.64 % of drug release in 12 hr and N5 shows 81.52 ± 2.56 % of drug release in 12 hr.

***IN VIVO* EVALUATION**

Figure 16: Experimental animals

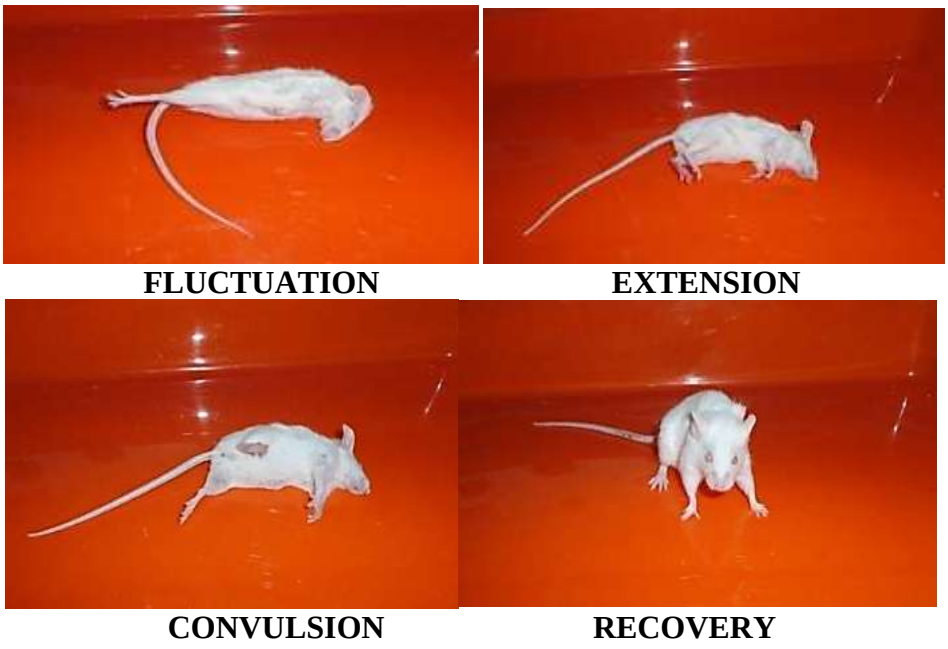
Species **:** Male albino mice
Sex **:** male
No of animals **:** 30
Body weight **:** 27-40g
Cages **:** 6 cages
Age **:** 8-10 weeks

Instrument used: Digital electro convulsometer

Figure: 17 Digital electroconvulsiometer

Data were expressed as mean values SEM and tested with variance analysis followed by the multiple comparison test of student t-test (one-tailed t-test)

MAXIMUM ELECTRIC SHOCK



FLUCTUATION **EXTENSION**

CONVULSION **RECOVERY**

Figure:18 various stages in producing convulsions by electro convulsiometer

Table no:8 Individual animal body weight of Electro Convulsiometer Induced Seizure

NO. OF ANIMALS / SEX	GROUP	BODY WEIGHT(gm.)
1/M	CONTROL (VEHICLE ONLY)	28.5
2/M		29.9
3/M		33.4
4/M		35.1
5/M		39.6
6/M		39.8
7/M	F-S9 TEST 1 (S6)	28.0
8/M		30.4
9/M		32.2
10/M		33.1
11/M		34.0
12/M		38.9
13/M		28.5
14/M		29.4
15/M	F-S9 TEST 2 (N5)	32.6
16/M		33.1
17/M		36.1
18/M		37.2
19/M		28.0

20/M		31.4
21/M		33.2
22/M	**REFERENCE CONTROL (EPTOIN TABLET)**	33.1
23/M		35.1
24/M		38.0

2. CONCLUSION

From the all the above date and potency of Anticonvulsant activity we can conclude that NLC is the best selected formulation to deliver anticonvulsant herbal drug like *Convoline.* Scope of future work it to identify the specific compound responsible for anticonvulsant activity from convoline and to overcome their pros and cons in converting into dosage form. To determine the pharmacokinetic and Pharmacodynamic studies in human volunteers and to apply for patent. From the research we Selected Suitable novel dosage form carrier molecule (NLC) was for low soluble and permeable herbal drugs like *Convoline,* by comparing the various data's between NLC and SLN. A novel NLC carrier was designed to incorporate herbal drugs that come under BCS class IV drugs, like low soluble and low permeable. From the research it was suggested that NLC nanocarrier was applicable to incorporate various herbal drug that shows low solubility and low permeability.

3. ACKNOWLEDGEMENT

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