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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/15007
DOI URL: http://dx.doi.org/10.21474/IJAR01/15007

RESEARCH ARTICLE

DESIGN AND DEVELOPMENT OF NANOSTRUCTURE LIPIDS CARRIERS FOR SOLUBILITY ENHANCEMENT OF CURCUMIN

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Manuscript Info

Manuscript History
Received: 05 May 2022
Final Accepted: 08 June 2022
Published: July 2022

Key words:-
Curcumin Based Nanostructured Lipid Carriers, Solubility Enhancement Of Pure Drug

Abstract

Low solubility of the drug limits its biological application so there is need to enhance the solubility and hence bioavailability of the drug. This review contains discussion about the formulation of nanostructured lipid carriers is one of the best method to increase the solubility hence bioavailability of the drug.

Introduction:-

Nanotechnology has become an essential element of pharmaceutical sciences and finds multiple applications in drug delivery to enhance the performance of the drug. It the study of design, creation, synthesis, manipulation and application of materials devices and systems at the nanometre scale such as 1 to 100 nm. Nano materials differ from other materials due to two major factors that are increased surface area and quantum effects. These factors can enhance properties such as reactivity, strength, electrical characteristics and in-vivo behaviour.[1] The technology can allow target delivery of drugs that are poorly water soluble and nanostructures able to penetrate tissue and are easily taken up by cells; allowing for efficient delivery of drug to the target site of action. To overcome the drawbacks associated to the traditional colloidal system such as emulsion, liposomes and polymeric nanoparticles, various nanotechnology techniques like NLC, Nanotubes, Quantum dots, Liposomes.(1, 2)

Pharmaceutical technology has taken the advantage of the advent of Nanotechnology. The term ‘‘Nanotechnology’’ was first used in a 1986 book, ‘‘Engines of Creation’’ by K. Eric Drexler. Nanotechnology is nothing but “the understanding and control of substances in the range of nanoscale”. Nanomaterial is defined as material which appears in one or more dimensions within range up to 100nm or less. The word “nanos” from nanotechnology is the Greek word with meaning dwarf. Nano- delivery systems are precious prospects for nanotechnology in pharmaceutics, cosmetics as well as food industries. Currently nanotechnology has widely used in the field of cosmetic so, consider as “hottest technology”. Now a day’s nano particles are incorporated in topical field because of their benefits such as increased efficiency, transparency, unique texture and protection of active ingredient so leads to higher consumer compliance.

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Nanoparticles are used in two categories:
i) Labile nanoparticles which get disintegrated into its molecular components when applied to skin (e.g. liposome's, micro-emulsion, nano-emulsion)
ii) Insoluble particles (e.g. Titanium dioxide, fullerenes and quantum dots.)

Poor water solubility and insufficient bioavailability of the new drug molecules are nowadays main problem. So to overcome this drawback a new drug carrier systems (such as Liposome's, nano emulsions, and lipid nanoparticles) are developed for controlled release of active substances and targeting to skin layers.(3, 4).

From these carrier systems the lipid based nano delivery system was developed i.e., Solid lipid nanoparticles (SLN) as an alternative carrier system to the existing traditional carriers such as liposome's, emulsions and polymer based nanoparticles.(4).

Solid lipid nanoparticles
SLN are introduced at the beginning of the 1990s and designed to combine the advantages of polymer particles, liposome's and emulsions to avoid their disadvantages. The SLN are produced from one solid lipid or a blend of solid lipid. The SLN are used as NDDS for pharmaceutical drugs in various application routes.(3, 4).

Nanostructured lipid carriers
NLC are modified Solid Lipid nanoparticles in which lipid phase contain the both solid lipid (fat) and liquid lipid (oil) at room temperature. It provides very useful information regarding the effect of drugs or excipients on the barrier function of skin. They are composed of physiological and biodegradable lipids as a carrier, exhibiting low systemic toxicity and low cytotoxicity.(5, 6,7)

Benefits of Nanostructured lipid carriers
1. Feasibility in incorporation of lipophilic and hydrophilic drugs.
2. Formulation can be done without the use of organic solvents.
3. No biotoxicity of the carrier.
4. Higher entrapment efficiency
5. Smaller size and low polymorphic changes.
6. Possibility of controlled drug release.
7. Increased drug stability.
8. High drug payload.

Nanotechnology has become an essential element of pharmaceutical sciences and finds multiple applications in drug delivery to enhance the performance of the drug. It the study of design, creation, synthesis, manipulation and application of materials devices and systems at the nanometre scale such as 1 to 100 nm. Nano materials differ from other materials due to two major factors that are increased surface area and quantum effects. These factors can enhance properties such as reactivity, strength, electrical characteristics and in-vivo behaviour.[1] The technology can allow target delivery of drugs that are poorly water soluble and nanostructures able to penetrate tissue and are easily taken up by cells; allowing for efficient delivery of drug to the target site of action. To overcome the drawbacks associated to the traditional colloidal system such as emulsion, liposomes and polymeric nanoparticles, various nanotechnology techniques like NLC, Nanotubes, Quantum dots, Liposomes.(1, 2)

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Structure of Nanostructured lipid carriers

The NLC mainly have three types of structure developed according to the formulation composition and the production parameters.

a) Imperfect Type:

Distances between fatty acid chains of glycerides and general imperfections in crystal are for the purpose of good drug accommodation. These imperfections accommodate drug in molecular form and it increase the drug payload.

b) Amorphous Type:

The crystalline process itself causes expulsion of the drug and forms NLC in amorphous form.

c) Multiple Types:

In this solid matrix of the lipid nanoparticles contains tiny liquid nanoparticles of the oil. These nano compartments are surrounded by solid lipid matrix thus allowing control released.

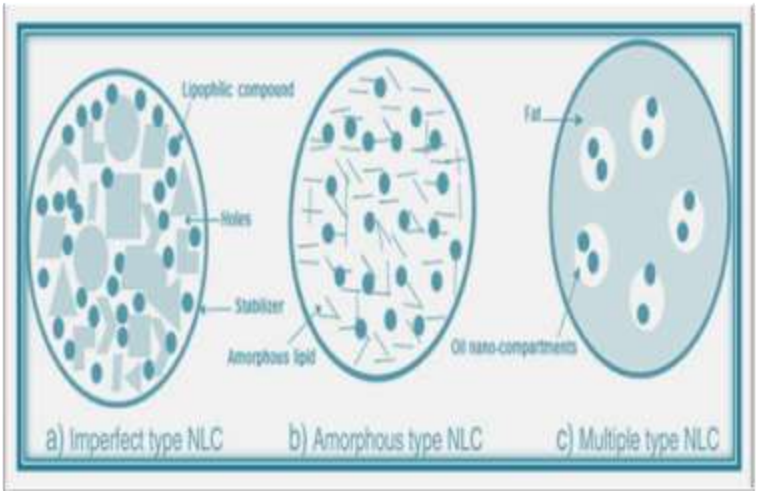


Figure1:- Structure of NLC

Materials used in the preparation of Nanostructured lipid carriers

The most commonly used ingredients for NLCs includes

1. Lipids = both solid and liquid lipids are included in NLCs for constructing the inner cores.
2. Emulsifiers = the emulsifiers have been used to stabilize the lipid dispersions.
3. Water

Methods for the preparation of Nanostructured lipid carriers

There are many methods for the preparation of lipid nanoparticles based DDS. The method used is dictated by the type of drug especially its solubility and stability, the lipid matrix, route of administration, etc.(1)

1. High pressure homogenization
2. Micro-emulsion technique
3. Solvent emulsification- evaporation technique
4. Solvent emulsification- diffusion technique
5. Phase inversion temperature method
6. Melting dispersion method
7. High shear homogenization or ultrasonication technique
8. Solvent injection technique
9. Double emulsion technique

High pressure homogenization, High shear homogenization and Microemulsification methods are considered to be standard for formulation of NLCs.

1) High Pressure Homogenization Technique

HPH has been used as a reliable and powerful technique for the large-scale production of NLCs. In High Pressure Homogenization technique lipid are pushed with high pressure (100-2000bars) through a narrow gap of few micron ranges. So shear stress and cavitations are the forces which cause the disruption of particle to submicron range. Normally the lipid contents are in the range of 5-10%. In contrast to other preparation technique High Pressure Homogenization does not show scaling up problem. Basically there are two approaches for production by high pressure homogenization, hot and cold homogenization techniques.For both the techniques drug is dissolved in the lipid being melted at approximately 5- 10º C above the melting point.**[1,65]**

a) Hot Homogenization Technique

In this technique the drug along with melted lipid is dispersed under constant stirring by a high shear device in the aqueous surfactant solution of same temperature. The pre-emulsion obtained is homogenized by using a piston gap homogenizer and the obtained nanoemulsion is cooled down to room temperature where the lipidrecrystallises and leads to formation of nanoparticles.[65]

b) Cold Homogenization Technique: -

Cold homogenization has been developed to overcome the problems of the hot homogenization technique such as, temperature mediated accelerated degradation of the drug payload, partitioning and hence loss of drug into the aqueous phase during homogenization. The first step of both the cold and hot homogenization methods is the same. In the subsequent step, the melt containing drug is cooled rapidly using ice or liquid nitrogen for distribution of drug in the lipid matrix as shown in the Figure 1.2. Cold homogenization minimizes the thermal exposure of the sample.[1,62

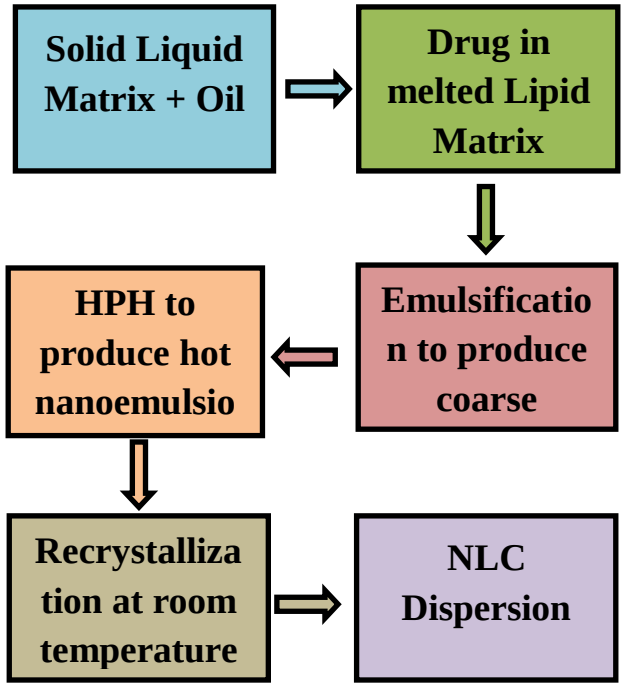


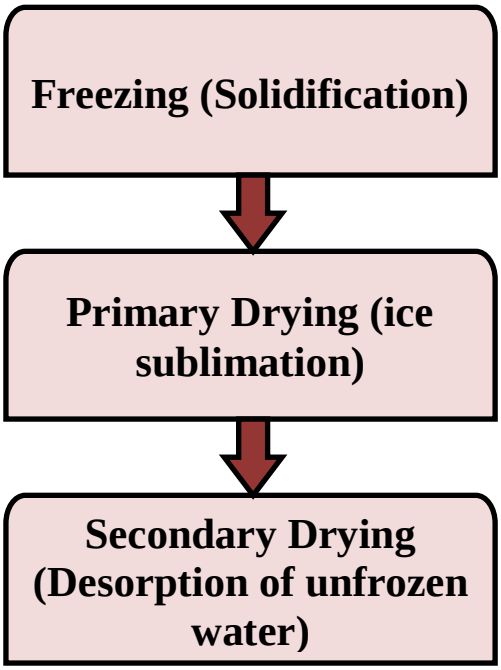
Figure 2:- Schematic procedure of hot homogenisation technique.

Lyophilisation(Freeze Drying)

The process of lyophilisation is most commonly used in industries for drying which allows transformation of solution or suspension form into solid dried powder of sufficient stability by removing water from frozen sample by sublimation and desorption under vacuum. Process of freeze drying allows transforming homogenized nanoemulsion into the soil powder state would prevent the Ostwald ripening and stop hydrolytic reactions.

Lyophilization cycle

Freeze drying cycle completed into three steps-



Characterization

A. Nanostructured Lipid carriers

1. Particle Size
2. Zeta potential (ZP)
3. Entrapment efficiency and drug loading
4. Dynamic light scattering. (DLS)
5. Atomic force microscopy (AFM)
6. Acoustic methods
7. Static light scattering
8. Scanning electron microscopy (SEM)
9. Transmission Electron Microscopy (TEM)
10. Differential scanning calorimetry (DSC)
11. X-ray diffraction
12. Nuclear Magnetic Resonance
13. Storage Stability
14. In-vitro and Ex-vivo methods

Application of Nanostructure lipid carriers:-

Topical drug delivery

Tacrolimus–loaded NLCs were successful prepared. The penetration rate of these NLCs through the skin of a hairless mouse was greater than that of Prototopic®. In vitro penetration tests revealed that the tacrolimus-loaded NLCs have a penetration rate that is 1.64 times that of the commercial tacrolimus ointment, Prototopic® . An increase of skin penetration was reported for coenzyme Q 10 (Q10)-loaded SLN compared to Q10 in liquid paraffin and isopropanol. The cumulative amounts of Q10 were determined performing a tape stripping test. After five strips the cumulative amount of Q10 was 1%, 28% and 53% of the applied amount from the liquid paraffin, the isopropanol and the SLN formulation, respectively. Similar results were achieved by another study for Q10- loaded NLC.(18, 37).

Cosmetics Application of NLC: -

Lipid nanoparticles i.e., NLC can be used to formulate active compounds in cosmetics. Incorporation of cosmetic compounds and modulation of release is more flexible when using NLC. In addition, sunscreen system based on tocopherol acetate has been described. A feature of general interest is the stabilization of chemically labile compounds. The solid matrix of the lipid nanoparticles protects them against chemical degradation, e.g. Vitamin E and coenzyme Q10. A recently discovered feature is the sunscreen blocking effect of lipid nanoparticles. Similar to particles such as titanium dioxide the crystalline lipid particles scatter UV light, thus protecting against UV irradiation. In addition, it was found that incorporation of sunscreens leads to a synergistic UV blocking effect of the particulate blocker lipid nanoparticles and the molecular blocker. In vitro, crystalline lipid nanoparticles with the same sunscreen concentration exhibited twice the UV protection effect compared with an O/W emulsion loaded with the sunscreen. (32).

Oral Drug Delivery:-

Interest in NLCs for oral administration of drugs has been increasing in recent years. Increased bioavailability, prolonged plasma levels are described for oral administration of NLCs. The lipid nanocarriers can protect the drugs from the harsh environment of the gastrointestinal tract. The lipophilic drugs can be entrapped by NLCs to resolve insolubility concerns. Atorvastatin, an anti-Hyperlipidemic agent with poor water solubility, has low oral bioavailability and a short half-life. [69]It is suitable to load into NLCs for improving oral delivery. Due to Instability of ifosfamide, NLCs of it is prepared to obtain sustain relese drug leakage during storage.(31)

Cardiovascular treatment

Lipid nanoparticles as a carrier system has superiorities mainly prolonged circulation time and increased area under the curve (AUC) with manageable burst effect. NLCs would provide highly desirable physic-chemical characteristics as a delivery vehicle for lipophilic drugs. Drug loading and stability were improved. Nifedipine loaded NLCs Nanoparticle suspensions were formulated with negatively charged phospholipid, dipalmitoylphosphatidyl glycerol in preventing coagulation to improve solubility and hence bioavailability of drug.

Drug delivery to brain

Brain targeting not only increases the cerebrospinal fluid concentration of the drug but also reduces the frequency of dosing and side effects. The major advantages of this administration route are avoidance of first pass metabolism and rapid onset of action as compared to oral administration. LNC (e.g. NLC) of this generation are considered to be one of the major strategies for drug delivery without any modification to the drug molecule because of their rapid uptake by the brain, bio acceptability and biodegradability. [73] In Bromocriptine loaded NLCs the In-vivo results showed bromocriptine NLCs have rapid onset of action and longer duration and higher brain levels as compared to that of solution, entrapment efficiency was also increased.

Ocular drug delivery

The characteristic features of NLCs for ocular application are the improved local tolerance and less astringent regulatory requirements due to the use of physiologically acceptable lipids. The other benefits include the ability to entrap lipophilic drugs, protection of labile compounds, and modulation of release behaviour. Recently, further investigations employing NLCs as ocular delivery systems have become known. In Cyclosporine loaded NLCs the mucoadhesive properties of the thiolated non-ionic surfactant Cysteine polyethylene glycol stearate (Cys-PEG-SA) and NLC modified by this thiolated agent were evaluated. Cys-PEG-SA and its resultant NLC provided a promising system with prolonged residence time. Lutein- loaded NLCs could protect the entrapped lutein in the presence of simulated gastric fluid and slowly released lutein in simulated intestinal fluid in an in-vitro study.

Pulmonary drug delivery

Inhalation drug delivery represents a potential delivery route for the treatment of several pulmonary disorders. NLCs have greater stability against the shear forces generated during nebulization compared to polymeric nanoparticles, liposomes and emulsions. NLCs are comprised of an inner oil core surrounded by an outer solid shell and hence allow the high payload of a lipophilic drug. Bioadhesiveproperties of NLCs are due to small particle size and lipophilic nature leads to longer residence time in lungs.

Cancer chemotherapy

In supplement, the function of NLC in cancer chemotherapy is presented and hotspots in research are emphasized. It is foreseen that, in the beside future, nanostructured lipid carriers will be further advanced to consign cytotoxic anticancer compounds in a more efficient, exact and protected manner. Loading of combiniedDoxoruibcinandcisplastin into NLC will increase synergistic anticancer effect,to maximize the treatment effect and multiresistnce. Loading of ZER into NLC will increase the bioavailability of the insoluble ZER in the treatment of cancers.l-arginine lauril ester (AL) into nanostructure lipid carriers (NLCs) and then coating with bovine serum albumin(BSA), pH-sensitive membranolytic and lysosomolyticnanocarriers (BSAAL-NLCs) were developed to improve the anti- cancer effect render more nanocarrierslysosomolytic capability with lower cytotoxicity, as well as improved therapeutic index of loaded active agents.

Intranasal drug delivery

The use of nanocarriers provides suitable way for the nasal delivery of antigenic molecules. These represent the key factors in the optimal processing and presentation of the antigen. Nasal administration is the promising alternative noninvasive route of drug administration due to fast absorption and rapid onset of action, avoiding degradation of labile drugs (peptides and proteins) in the GI tract and insufficient transport across epithelial cell layers. The development of a stable nanostructured lipid carrier (NLC) system as a carrier for curcumin (CRM) biodistribution studies showed higher drug concentration in brain after intranasal administration of NLCs than PDS. The results of the study also suggest that CRM-NLC is a promising drug delivery system for brain cancer therapy [80].In addition; NLC further enhanced the intranasal drug delivery of duloxetine in the brain for the treatment of major depressive disorder.

Parenteral drug delivery

The nano-drug delivery systems such as nanomicelles, nanoemulsions and nanoparticles has displayed a great potential in improved parenteral delivery of the hydrophobic agents since last two decades. NLC has been considered as an alternative to liposomes and emulsions due to improved properties such as ease in manufacturing, high drug loading, increased flexibility in modulating drug release profile and along with these, their aqueous nature and biocompatibility of the excipients has enabled intravenous delivery of the drug with passive targeting ability and easy abolishment. Another reported example is NLCs of all-trans Retinoic acid(ATRA) that offers significant

improvement in the anti-cancer activity and duration of action as compared to the conventional injectable formulation.

Materials And Methods:-

Materials:-

Curcumin was obtained from Bio Med Ingredients PvtLtd Mumbai, Maharashtra. Glycerylmonosterate was obtained from Alpha Chemicals Pvt Ltd Mumbai. Sunflower oil was obtained from Local market Wardha, Maharashtra. Tween -80 and Poloxamer 188was obtained from LOBA Chemicals Pvt Ltd Mumbai. Mannitol was obtained from S. D. fine Chemicals, Mumbai. All other reagents used were of analytical grade.

Methods:-

Glycerylmonosterate is used as solid lipid and sunflower oil is used as liquid lipid which is selected on the basis of lipid screening test. Tween 80 and poloxamer 188 were used as surfactant and stabilizer respectively. Curcumin based NLCs were prepared by High Pressure Homogenization (HPH). The Nanostructured Lipid Carriers are prepared by using hot homogenization and Lyophilization technique.

Accordingly, the weighed amount of drug (Curcumin) was added to the mixture of lipids i.e., solid lipid (GlycerylMonosterate) and liquid lipid (Sunflower oil) which was heated at 10 -15^{0}C above the melting point of solid lipid to this mixture co-surfactant (Poloxamer 188) was added and simultaneously, aqueous surfactant (Tween 80) solution was heated at the same temperature (85^{0}C).

Then the lipid mixture was poured in the hot aqueous surfactant solution using a magnetic stirrer (Remi instruments Ltd., Mumbai, India) at 12,000 rpm for 30 min,to prepare the primary emulsion. This primary emulsion was converted to the NLC system using high pressure homogenizer (KinematicaSPolytron, PT 3100 D) at 10,000 rpm for 60 min. The obtained NLC dispersion was cooled down to room temperature. The NLC dispersion was lyophilized by using lyophilizer (Lark Inovative Penguin classic plus 4 KG) for long term stability. Mannitol (3% w/v was added as cryoprotectant. The 3 different batches are prepared as follows;

CUR – NLC	Glycerylmonosterate (% w/v)	Sunflower oil (%w/v)	Tween 80 (% w/v)	Poloxamer 188 (% w/v)	Drug-CUR(mg)	Water (Upto 100 ml)
NLC 1	0.9	0.1	0.2	01	10	QS
NLC 2	1.35	0.15	0.2	01	10	QS
NLC 3	1.8	0.2	0.2	01	10	QS

Table No- 1:- The Composition of the Curcumin based NLCs.

Characterization of Nanostructured lipid carriers

Particle Size &polydispersity index[1,10,12,13]

The particle size is important parameter in process control and quality assurance because physical stability of vesicle dispersion depends on particle size and as particle size decreases, surface area characteristics increases as a function of total volume. The mean diameter (z-average diameter) and size distribution were measured by photon correlation spectroscopy (PCS) (Nano ZS Zetasizer, Malvern Instruments Corp, UK) at 25^{0}c in polystyrene cuvette with path length of 5 mm at an angle of 90^0. Nanostructured lipid carriers were suspended in double distilled water and one drop was placed on clean slide and the particle size was observed.

Zeta potential[1,12,13]

Zeta potential is a parameter which is very useful for the assessment of the physical stability of colloidal dispersions. The surfaces of particles develop a charge due to ionization of surface groups or adsorption of ions. This charge depends on both the surface chemistry of the particles and the media around these particles. The surface charge generates a potential around the particle, which is at the highest near the surface and decays with distance into the medium. The zeta potential can be measured by determining the velocity of the particles in an electrical field (electrophoresis measurement). The zeta potential was measured in capillary cells with path lengths of 10 mm, using the Nano ZS Zetasizer. Measurements were performed in distilled water obtained by a MilliQ system.

Fourier Transfer Infrared Spectroscopy :-[11,12,13]

Drug excipients compatibility study was done by FT-IR. To study the interaction between drug and carriers used in the preparation of NLC. FT-IR spectrum of pure drug: curcumin, physical mixture of drug: lipid (stearic acid) and Optimized formulation NLCs was recorded. The drug and carriers separately and in combination with each other were mixed with KBr (IR grade) in the ratio of 1:100. The pressure of 15000 lb was applied in hydraulic press. The scanning range selected was from 400cm-1 to 4000cm-1 in FT-IR Spectrophotometer (Shimadzu, Japan DR-8031). Spectra of mixture of curcumin and lipid were compared with spectrum of curcumin for any interaction.

Differential scanning calorimetry[1,12,36]

DSC is usually used to get information about both the physical and the energetic properties of a compound or formulation. DSC measures the heat loss or gain as a result of physical or chemical changes within a sample as a function of the temperature. The rate of crystallinity using DSC is estimated by comparison of the melting enthalpy/g of the bulk material with the melting enthalpy/g of the dispersion. The DSC thermo grams of the drug and lyophilized NLC was recorded using instrument DSC Q20 V24.11 Build 124 at the scan rate 10^0/min from 30 to 250^0c.

X-ray diffraction studies[8,13]

XRD studies of pure drug (CUR) and NLC formulation were done to find out the change in crystallinity when drug was mixed with Stearic acid lipid matrix. XRD pattern were recorded using (Bruker, D8) with Cu-kα radiation. The scanning angle ranged from 3° to 50° of 2θ.

Scanning electron microscopy[01,12,13]

The surface morphology of NLC was observed by SEM. NLC loaded with drug were fixed on a stub using double-sided adhesive tape and then made electrically conductive by coating with a thin layer of gold for 30 sec using JEOL fine coat (JFC-1100F ion sputtering device) and scanned using JEOL(JSM-6360) SEM at30.0 KV.

Solubility determination[13,14]

The solubility of drug and prepared batches of NLCs was determined by taking an excess amount of drug and adding it to 10 ml of solvent (Distilled water), in Teflon-facing screw-capped vials. The samples were kept at equilibrium for a period of 24 h on an orbital shaking incubator (CIS-24; Remi Instruments, Mumbai, India) at 37±0.5°C and 50 rpm. The supernatant liquid was collected and filtered through 0.2µ membrane filter and analyzed by UV visible (Shimadzu, Japan UV-1800) at wavelength 426 nm for Curcumin.

Entrapment efficiency and drug loading[1,13]

The entrapment efficiency (%) and drug loading was determined by measuring the concentration of free and entrapped CUR. A known dilution of the NLC dispersion was prepared and 1ml was transferred to the upper chamber of centrifuge tubes of REMI motor centrifuge for 30 min. Centrifuge tubes were centrifuged at 10,000 rpm for 30 min. The filtrate was analyzed for un encapsulated curcumin at 426 nm using a validated UV-Spectrophotometric method after suitable dilution. Entrapment efficiency and drug loading was calculated by following formula.

In-vitro dissolution of Curcumin and NLC[1,13]

In-vitro dissolution studies of curcumin Nanostructured lipid carriers were carried out by USP type rotating basket type dissolution apparatus (Electro lab, Mumbai). Optimization of formulation batches was estimated on the basis of cumulative percentage drug release with respect to time. The dissolution carried out in two different media distilled water and phosphate buffer saline (pH 6.8) each of 900 ml. NLCs were placed in each vessel and the medium was allowed to maintain at 100 ppm at 37^0c± 0.5^0c. Samples of 10 ml were withdrawn at various time intervals up to 24 hr and sink condition was maintained. The absorbance of sample was measured by UV double beam spectrophotometer (Shimadzu, Japan UV-1800) at 421 nm and cumulative percentage drug releases were calculated.

Results and Discussion:-

Particle Size &polydispersity index

The optimized NLCs were in the nanometric size range (183.01 nm) with low polydispersity index 0.706 (fig 4.13). The presence of 30% of oil in the NLC lipid matrix led in to small mean diameter of NLC. Surfactant also greatly influences the particle size of formulation by causing stabilization. The nano size may be the reason for enhanced solubility of drug.

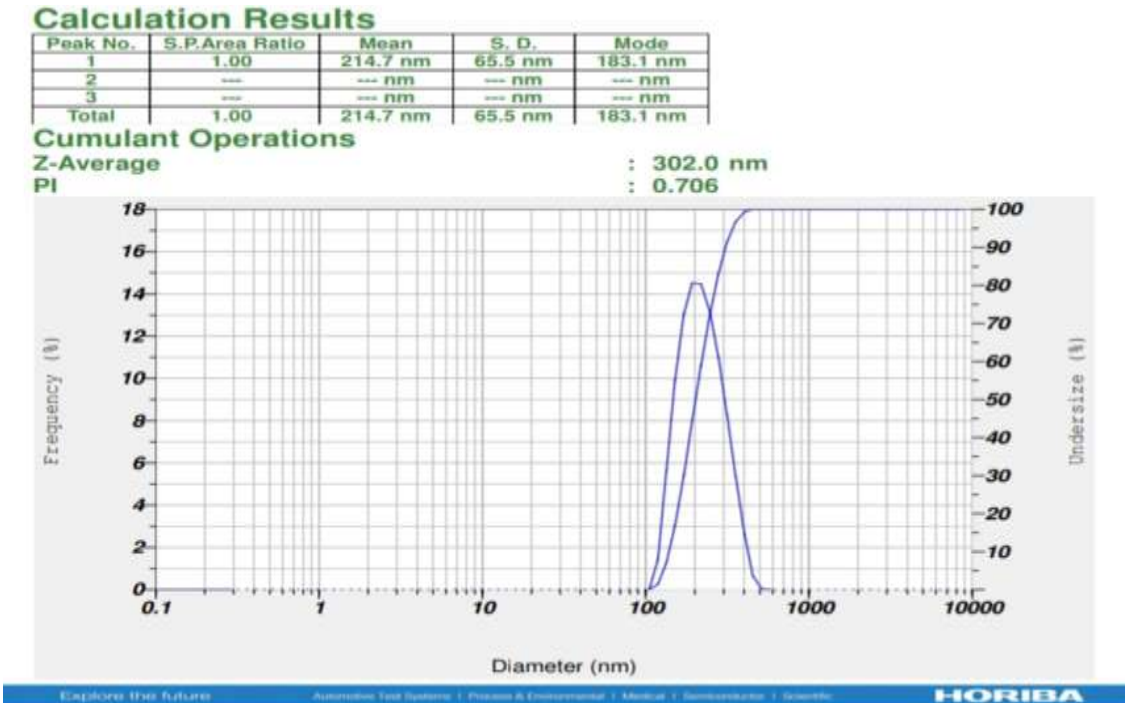


Figure No 3:- The particle size and particle size distribution of optimized formulation NLCs.

Zeta potential

The zeta potential of the optimized formulation was found to be 31.1mV. Which is the indicative of the stability of the formulation.

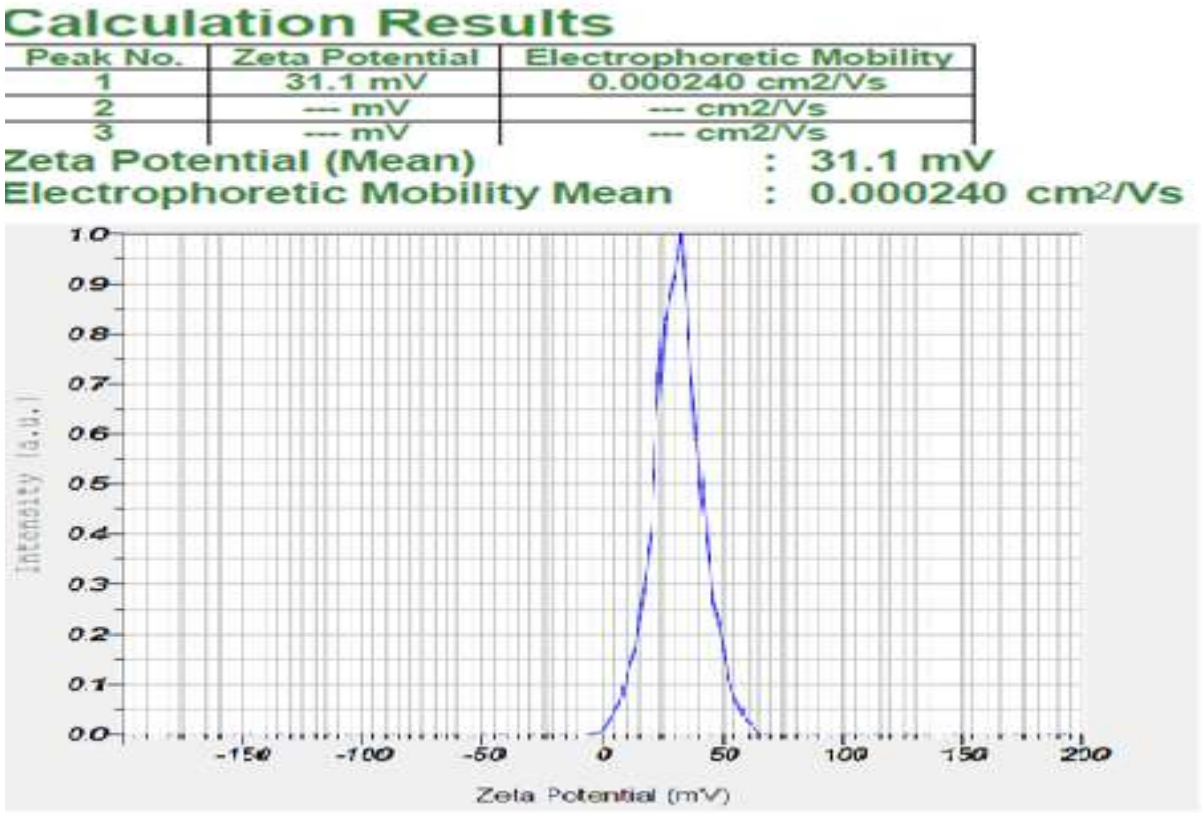


Figure No 4:- The Zeta potential of optimized formulation NLCs.

FT-IR

The FT-IR studies are done to check that what changes are done of drug with lipid. FT-IR studies of pure Curcumin Drug (CUR), Solid lipid (Glycerylmonosterate), Liquid lipid (Sunflower oil) and Surfactant (Tween-80) physical mixture Optimized batch (NLC) were performed. The FT-IR Spectrums of pure Curcumin Drug (CUR), Solid lipid (Glycerylmonosterate), Liquid lipid (Sunflower oil) and Surfactant (Tween-80) and physical mixture optimized batch (NLC) were shown in fig 5,6,7,8 & 9, respectively.

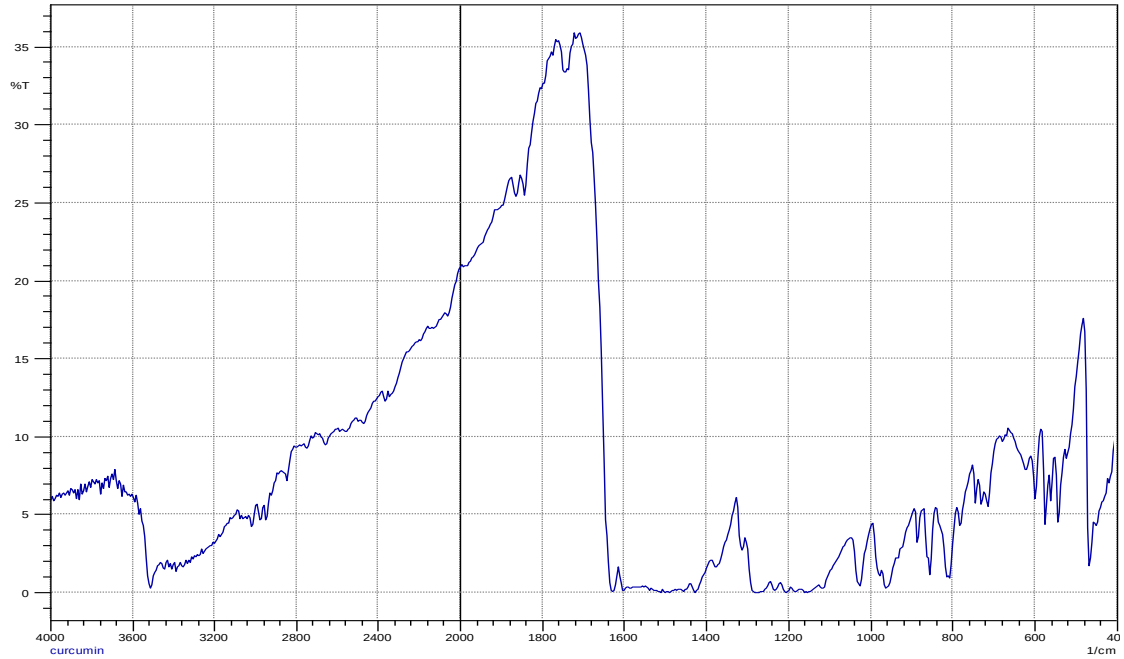


Figure No 5:- FT-IR spectrum of pure drug Curcumin (API).

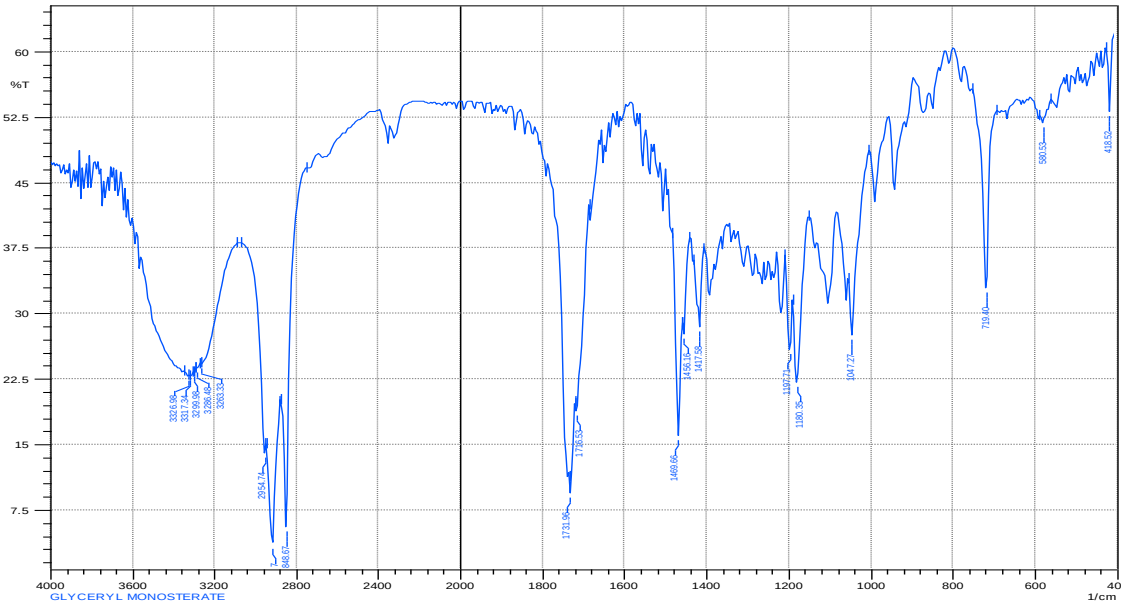


Figure No 6:- FT-IR spectrum of GlycerylMonosterate (Solid Lipid).

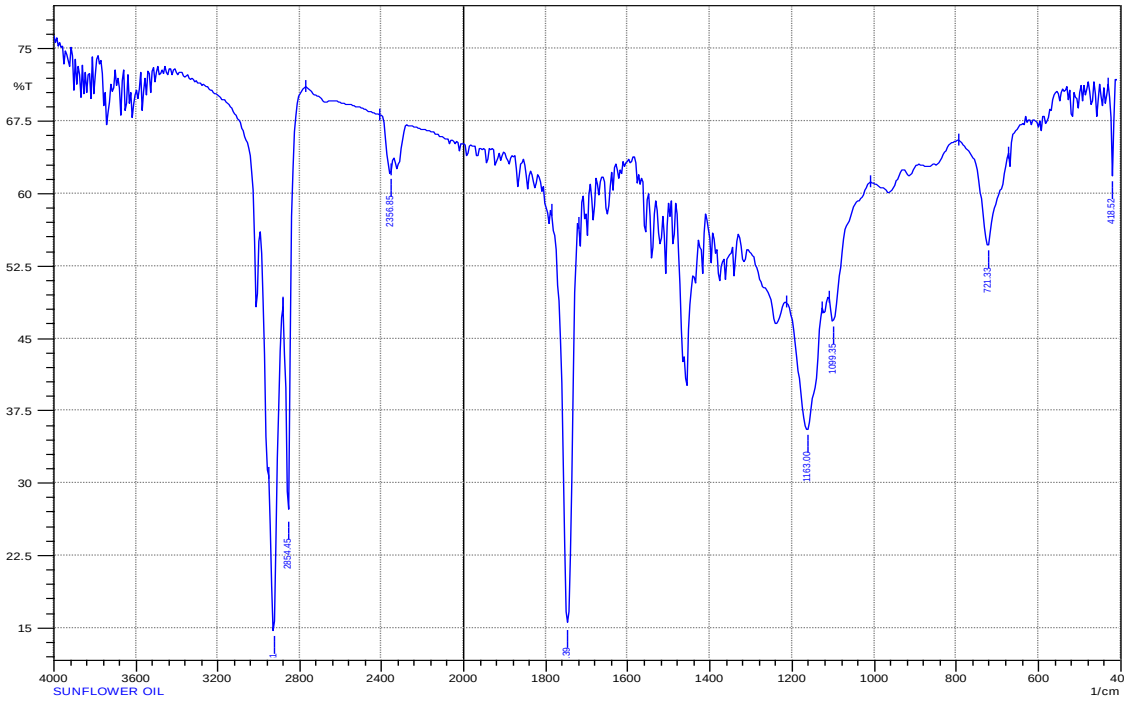


Figure No 7:-FT-IR spectrum of Sunflower oil (Liquid lipid).

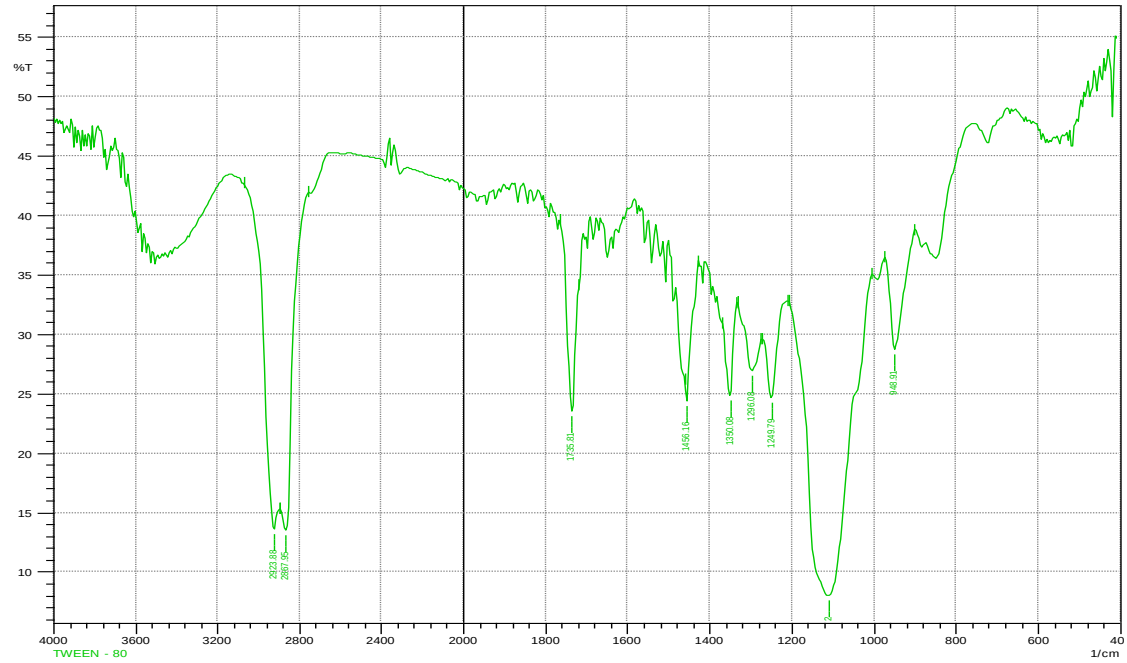


Figure No 8:- FT-IR spectrum of Tween-80 (Hydrophilic Surfactant).

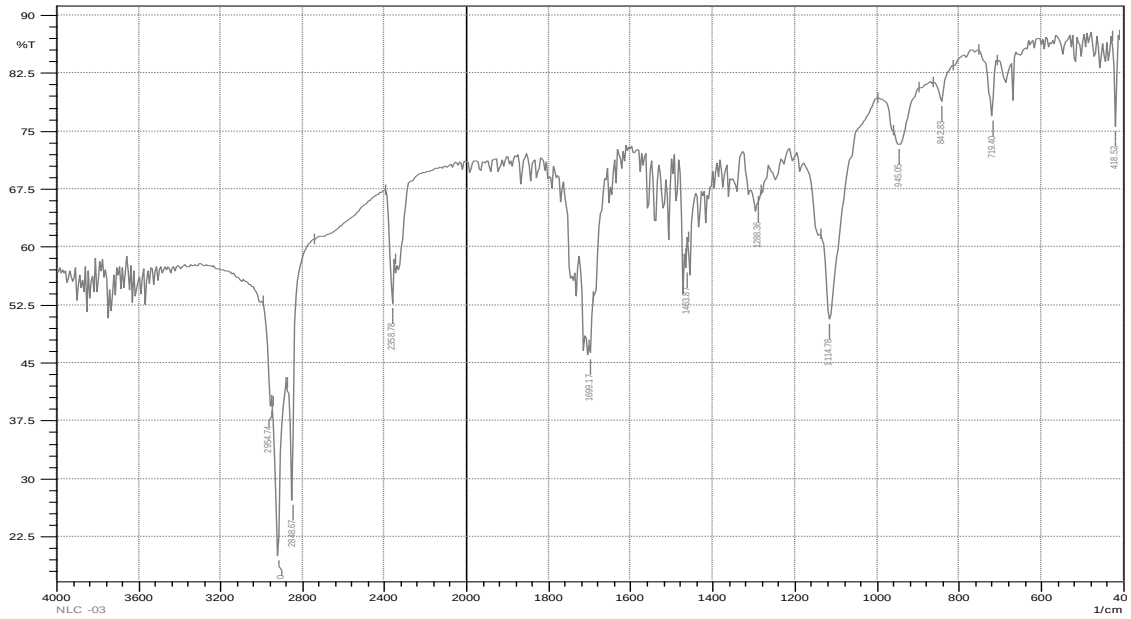


Figure No 9:- FT-IR spectrum of optimized batch NLC.

The obtained peaks are of functional group of material when compared with standard IR frequency range of functional group, it confirms the originality of material. curcumin drug shows principle peaks at 3470, 3110.97, 1627.81, 3012.6 and 1604.66. From above ranges it can be concluded that drug and excipients are compatible with each other.

Differential Scanning Colorimetry

Indicating Curcumin was essentially encapsulated into the lipid with amorphous state. A shift in the peak was seen (fig11) which may be due to the interaction of the drug with lipid matrix. The DSC thermogram suggests that the drug is completely enclosed inside the NLC. The melting point peak is found at the temperature 186.54^0c to that of the NLCs i.e. 175.56^0c.

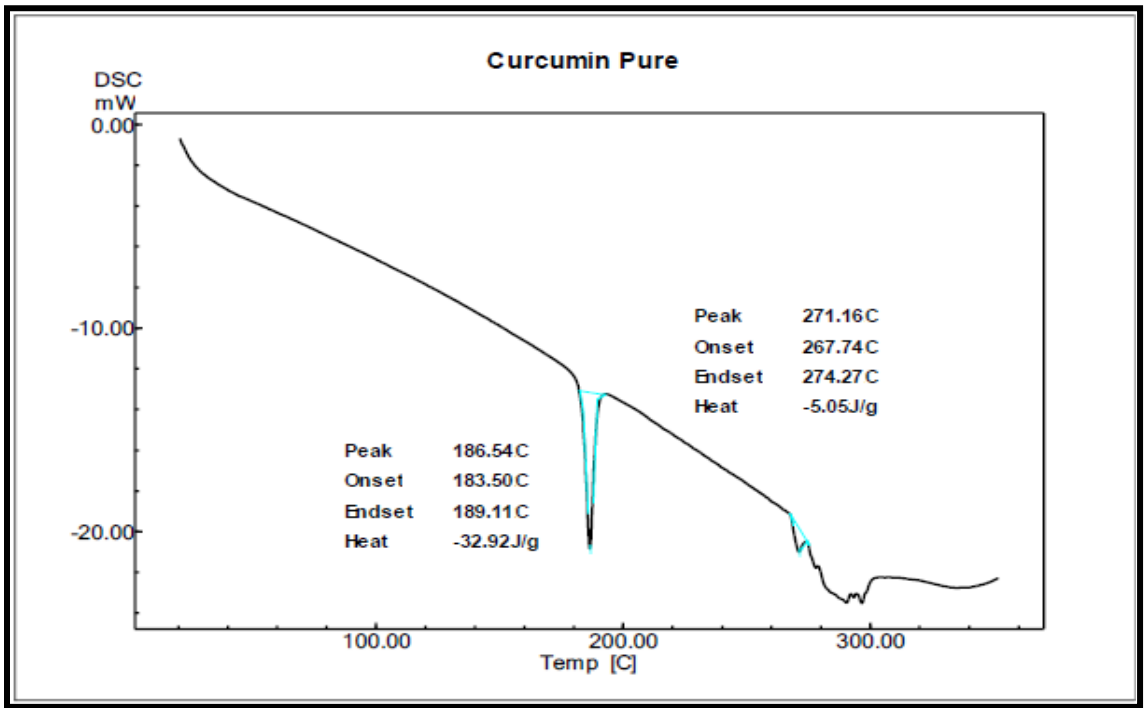


Figure No 10:- DSC thermogram of pure drug Curcumin.

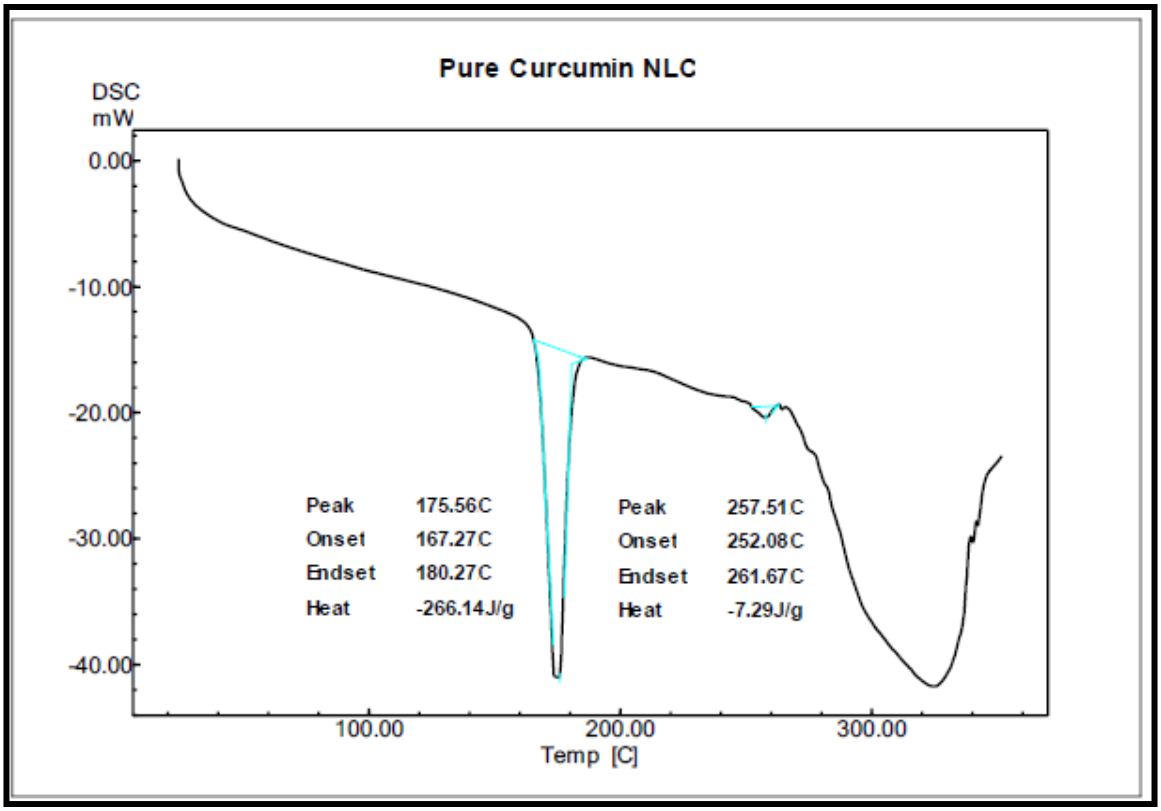


Figure No 11:- DSC thermogram of optimized formulation NLC 9.

X-ray diffraction studies

Curcumin powder was highly crystalline as evident from sharp peaks seen at the 2θ value in the x-ray scan (fig 12). Characteristic diffraction peaks at 8.50^0, 17.16^0, 25.50^0, and 24.50^0 were observed with intense peak at 24.50^0. Fig 13 shows the XRD patterns of freeze-dried Curcumin loaded NLC showing that peak intensity is reduced indicating reduction in crystallinity. The absence of these characteristics reflections in lyophilized NLCs demonstrated the total solubilization of drug within the lipid phase

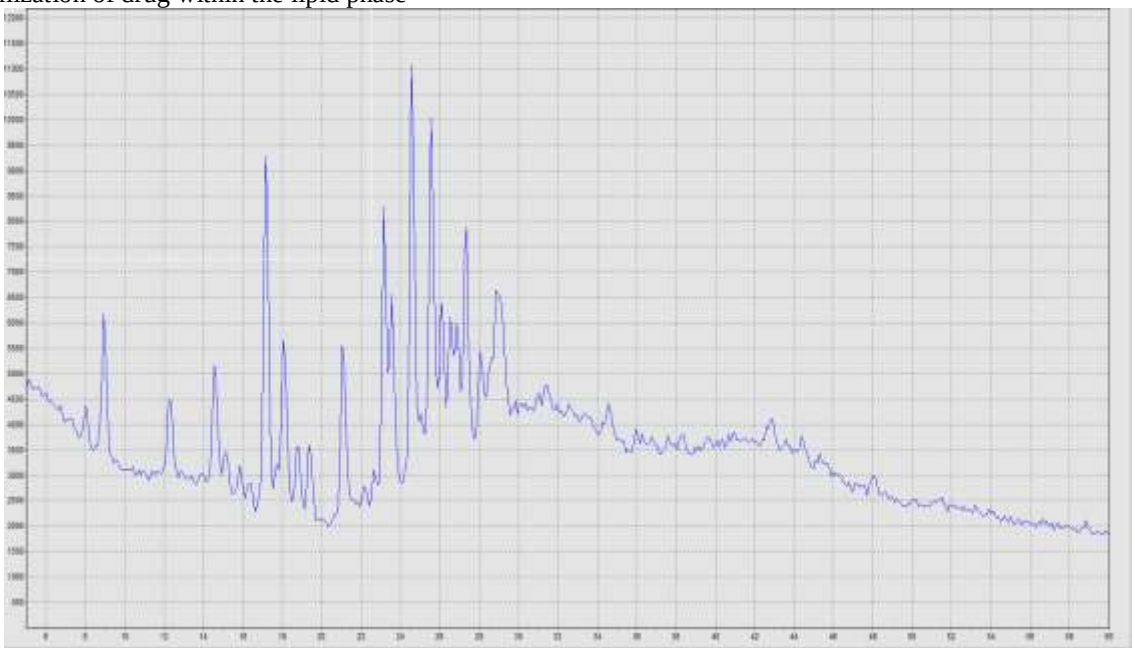


Figure No 12:- XRD graph of pure drug.

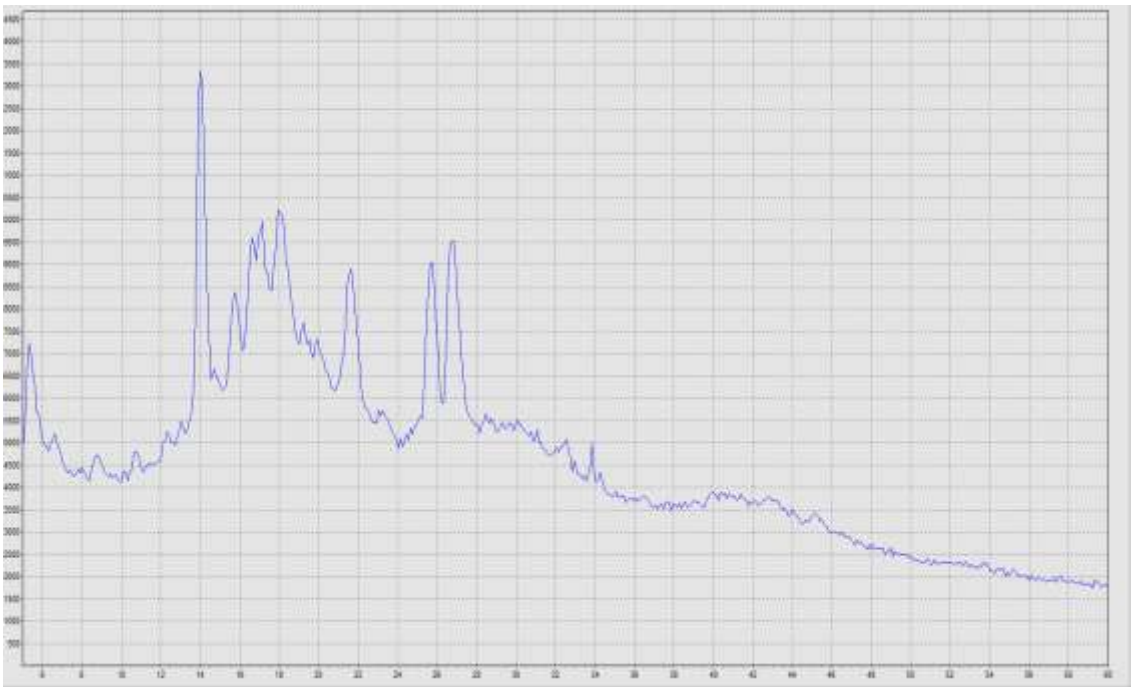


Figure No 13:- XRD graph of NLC 3.

Scanning electron microscopy

The SEM of Curcumin and NLC 3 was shown in fig 14. From the fig A it is clearly seen that Curcumin particles were irregular shaped smooth and rough surface, while in case of fig B the NLC particles are found to be spherical shape. This change is due to the entrapment of the drug into the lipid matrix.

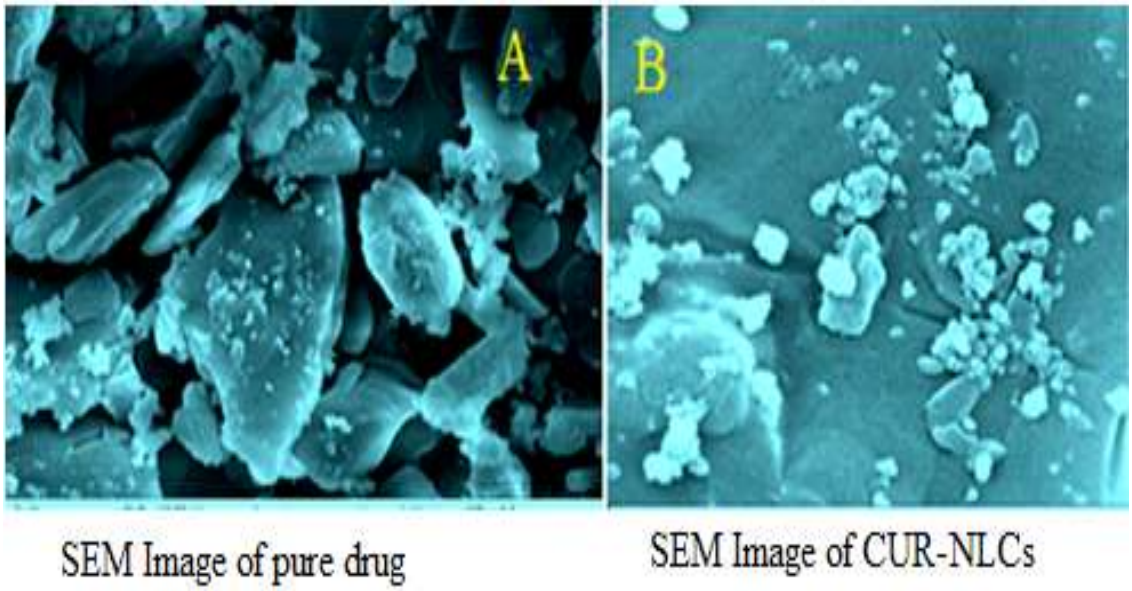


Figure No 14:- SEM images of pure drug Curcumin (A); NLC (B).

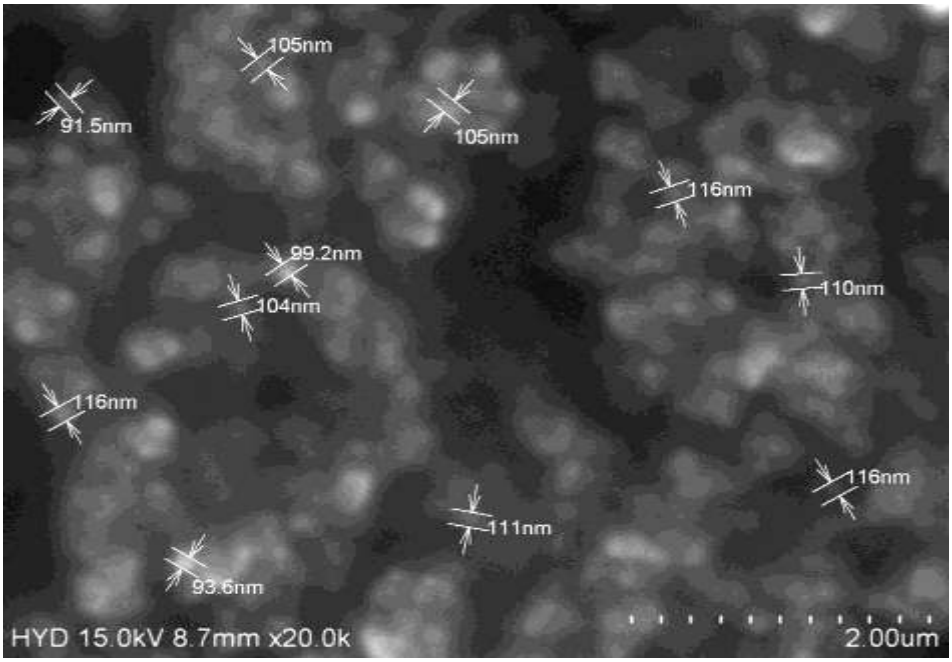


Figure No 15:- SEM images of NLC 09.

Solubility determination

Drug &NLCs	Solubility in mg/ml
Drug (Curcumin)	0.0049 ± 0.007
NLC 1*	0.1143 ± 0.046
NLC 2*	0.3514 ± 0.073
NLC 3*	0.6735 ± 0.083

Table No 2:- Solubility of drug (Curcumin) & optimized NLCs.

Entrapment efficiency and drug loading

Percentage entrapment efficiency is indicative of the percentage of the drug that has been encapsulated within the lipid NLCs with respect to the feed drug concentration. The percentage encapsulation efficiencies and drug loading were calculated by indirect method as per the formulae mentioned in the experimental section. The result of percentage entrapment efficiencies is as shown in Table. It was found that Curcumin could be entrapped with high efficiencies in the NLC-9 formulation due to its lipophilic nature. The average entrapment of Curcumin in the formulated NLCs was found to be 55.48 ± 0.246% as calculated in triplicates.

Formulation	% Entrapment efficiency	% Drug loading
NLC 1	31.27 ± 0.356	13.39 ± 0.315
NLC 2	41.72 ± 0.312	14.34 ± 0.308
NLC 3	55.48 ± 0.246	16.27 ± 0.345

Table No 3:- % Entrapment efficiency & % Drug loading of NLCs.

In-vitro dissolution of Curcumin and NLC

In vitro dissolution of Nanostructured Lipid Carriers were carried out by using USP Apparatus type XXIV (TDT 08L Electro lab, Mumbai, India) at 100 rpm in two different media phosphate buffer saline pH 5.7. The results of in vitro dissolution of Nanostructured Lipid Carriers were shown in table 4.

Table No 4:- In-vitro dissolution of NLCs in phosphate buffer pH 5.7.

Time	NLC 3 (% CDR)	NLC 6 (% CDR)	NLC 9 (% CDR)

00 min	00	00	00
05 min	0.304 ± 051	0.307 ± 0.59	0.309 ± 0.50
15 min	2.828 ± 0.50	3.471 ± 0.72	3.394 ± 0.97
30 min	5.914 ± 0.65	9.516 ± 0.75	8.871 ± 0.79
45 min	9.387 ± 0.60	16.33 ± 0.79	17.125 ± 0.65
1.0 HR	15.84 ± 0.43	23.99 ± 0.65	26.285 ± 0.72
02hr	22.78 ± 0.69	31.70 ± 0.72	35.933 ± 0.42
04 hr	30.44 ± 0.56	39.67 ± 0.67	46.78 ± 0.65
06 hr	38.67 ± 0.88	48.03 ± 0.72	56.78 ± 0.52
08 hr	48.90 ± 0.59	57.14 ± 0.79	67.30 ± 0.80
10 hr	59.70 ± 0.66	67.30 ± 0.90	78.36 ± 068
12 hr	71.66 ± 0.80	77.55 ± 0.57	89.55 ± 0.95

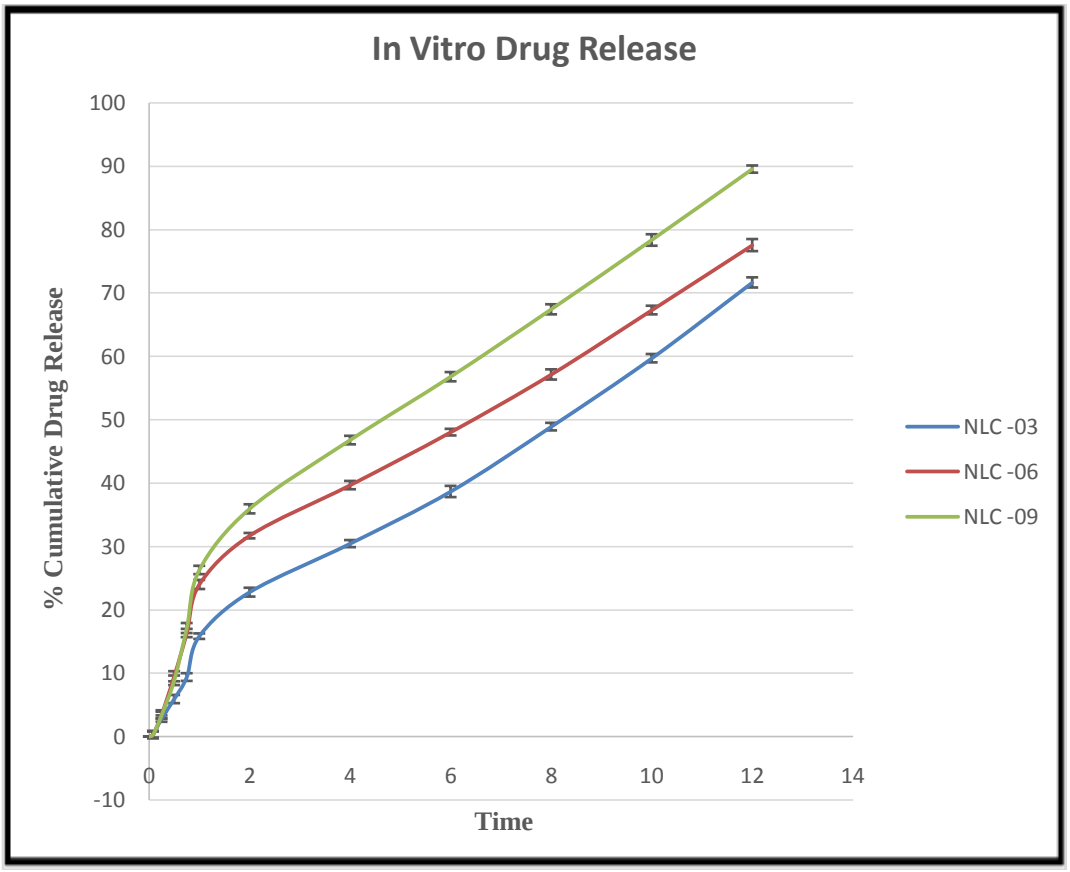


Figure No 16:- In-vitro dissolution of NLCs in phosphate buffer pH 5.7.

Conclusion:-

In the present study of curcumin loaded Nanostructured lipid carriers were successfully prepared using hot homogenization (High pressure homogenization) and Lyophilization (Freez drying) technique.

The prepared Nanostructured lipid carriers were found to have enhanced solubility.

The optimized batch were with uniform particle size and show better drug entrapment efficiency, drug content and in vitro release. The physical characterization of optimized batch (Curcumin based NLCs) via.

DSC and XRD showed the presence of curcumin and also transfer crystalline curcumin into amorphous curcumin.

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