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DEVELOPMENT OF NANOSTRUCTURED LIPID CARRIER SYSTEM OF QUETIAPINE FUMARATE AND CURCUMIN TO TREAT SCHIZOPHRENIA BY CENTRAL COMPOSITE DESIGN -DOE APPROACH AND EVALUATION BY MOLECULAR DOCKING:IN SILICO METHODS.

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Abstract:

The work aims at formulating nanostructured lipid carrier (NLC) of quetiapine fumarate in combination with curcumin to treat schizophrenia. The prominent scope is to optimize the concentration of lipids by design of experimental approach (DOE) and to formulate a stable nanostructured lipid carrier system. The lipids incorporated in this were cholesterol as solid lipid and oleic acid as liquid lipid, vitamin E TPGS plays its role as solublizer, stabilizer and a non - ionic surfactant. The formulations were optimized by response surface method. The 2^2 factorial design is employed for the sake of achieving the desired goal. NLC were prepared by hot homogenization followed by ultrasonication. The NLC formulations were subjected to diversified studies that comprises of entrapment efficiency, invitro release studies, size analysis, surface morphology and stability studies. The molecular docking analysis to know the binding of the formulation with D2 and DAT receptors. All the parameters of the study resulted in satisfactory results with the optimized formulation (QC-NLC-1) having particle size of 93nm with entrapment efficiency of 97.8% and invitro release rate of 93.7% of quetiapine fumarate and 63.59% of curcumin in 72 hours. The formulation was found to be stable with all its characteristics retained even after 180 days at 40±2°C/ 75±5% RH. In silico studies by molecular docking and ADMET revealed the docking of drug at various receptor level ensuring the formulated NLC penetrates BBB. In case of D2R, curcumin and quetiapine showed considerable binding affinity with free binding energies of -5.76 and -5.52 Kcal/mol respectively and had sufficient hydrogen bonding with receptors.

Keywords: Nano structured lipid carrier, quetiapine fumarate, curcumin, schizophrenia, Vitamin E TPGS, in silico methods, molecular docking.

Introduction:

Schizophrenia is knotty disorder affecting men and women all over world. As per recent hypothesis the hyper active and hypo active transmission of dopamine in certain areas is the cause for schizophrenia. This chronic neuro psychiatric disorder results in diminished cognitive

flexibility as the major symptom. Hallucinations, delusions (said to be positive symptoms) strange dangerous attitudes, anti-social behaviour, suspiciousness, in coherent speech (negative symptoms). [1] The schizophrenia which is a complex disease due to many factors contributing. The positive symptoms are due to augmented release of dopamine in subcortical areas resulting in D_2 receptor activation and negative symptoms are because of decreased D_1 receptor activation.[2] Apart from dopamine contribution, the disease may also from other neurotransmitters. Another excitatory neurotransmitter which plays major role in brain development in memory and thinking process is glutamate. As per glutamate hypothesis, diminished function of the glutamate in certain regions like cortico striatal projections leads to an opening effect in the thalao-cortical loop causing an augmented sensory flooding causing a change in psychotic symptoms followed by dopamine concentration changes. The CSF of schizophrenic patients revealed decreased concentration of glutamate. The negative and cognitive impairments associated with this is due to dysfunction glutaminergic neuro transmission.[3] In nut shell the reason for the aliment lies on different theories /hypothesis of neurotransmitters like hyper activeness of dopamine, Diminished function of excitatory neurotransmitter glutamate, accumulation of GABA in certain areas of brain.

The contributing factors are not only the neurotransmitters but genetic factors, lifestyle patterns also play crucial role. The first-generation antipsychotics showed better effect after blocking dopamine receptors, a new ray of hope arises in field of psychiatric medicine. The complex disease arises in young adult stage in both sexes but the risk seems to be higher in men. The illness is characterized by schizophrenic illness, delusions, distorted thinking, inappropriate perceptions and hallucinations. As per WHO the disease is eighth leading cause of disability adjusted life years (DALY)[4]

Antipsychotic medications, atypical antipsychotics are the drug of choice in the treatment of schizophrenia preferred to typical medicines. In midst of atypical antipsychotics, certain drugs face flaws like low oral bioavailability, extensive hepatic metabolism, low half-life etc., these lacunae can be masked by a means of targeted drug delivery system by intra nasal drug delivery [5,6]

The birth of nanotechnology as implanted its feet in medical field paving way for nano formulations to target certain ailments. A complex task in mitigating the CNS disorders is penetration of drug through blood brain barrier system to achieve the target site. The only way to reduce the side effects of the drug is by targeting the drug delivery to the site of action thereby the distribution of drugs to the other organs is diminished. The main barrier that exists as challenging to this is the Blood brain Barrier. A lot of approaches are there to bypass this barrier and to acquire desired concentration in brain. In the recent years there is a booming growth in the field of nano medical science. The main challenging in brain related disorders is crossing blood brain barrier which is complex structure.

The birth of nanostructured lipid carrier system has given a new ray of hope in delivering drugs to these patients suffering from schizophrenia, mania and other mood disorders termed as psychic disorder. The nano particulate drug delivery system has proved to be efficient because of its size, tailored surface, solubility improvement and targetability. Nanoparticulate drug delivery

may be either from polymer based or lipid based. Among them lipid based carriers are biodegradable, less toxic, rapid uptake by brain. Solid lipid nanoparticles(SLN) and Nano structured lipid carrier systems (NLC) are the two leading lipid carrier systems . As colloidal carriers, polymeric nanoparticles, solid lipid nano particles failed to cement certain cracks in formulation, stability and delivery of the drug. The NLC is dominant over SLN and other carrier systems. Recent budding area of nanostructured lipid carrier is blossoming in the field of nano era to treat many aliments [7-10]

Quetiapine fumarate is one of the atypical antipsychotic drugs belonging to dibenzothiazepine derivative. It is used in treating schizophrenia and bipolar disorder. Among the atypical antipsychotics available, the superior therapeutic efficacy and cognitive properties were observed in quetiapine fumarate and hence selected for experimenting.[11] Being BCS class II drug it has only 9% oral bioavailability. Being P-gp (P-glycoprotein) substrate the entry of quetiapine fumarate into the brain is restricted. Hence it is determined to include curcumin in the formulations as curcumin is an inhibitor to P-gp, also assist in managing schizophrenia apart from inhibiting P-gp thereby achieving the drug targeting. Curcumin also acts as P-gp modulator hence used as carrier in the formulation. [12]

Curcumin is a lipophilic compound which has ability to penetrate BBB. The earlier reports and research suggest that curcumin ameliorate the extra pyramidal and metabolic side effects when given as adjuvant with anti-psychotic drug. Its major mechanism underlies on regulation of oxidative stress and inflammation, reduction of mono aminogeric and hypo thalamo pituitary adrenal axis disturbances, limiting mitochondrial dysfunction and hindering worsening of neuro psychotic disorders [13-15]

The work focuses on formulating NLC of quetiapine fumarate and curcumin with an idea of reducing dose of the drug by sustaining the effect and to improve the stability with vitamin E TPGS as surfactant. A water-soluble derivative and the natural source of vitamin E is D-α-tocopheryl polyethylene glycol (PEG) 1000 succinate (TPGS). This substance apart from being amphipathic and hydrophilic, it is also a typical surface- active agent. It is widely used as emulsifier and stabilizer in formulation of nanoparticles. [16]

MATERIALS:

Quetiapine fumarate was obtained as gift sample from Dr. Reddys laboratories Ltd, Hyderabad, India. Curcumin from natural remedies, Bangalore, India. Potassium dihydrogen phosphate, sodium hydroxide, acetonitrile, methanol, Cholesterol, Vitamin E TPGS were from SD fine chemicals. All the chemicals used were of analytical grade.

FORMULATION OF NANOSTRUCTURED LIPID CARRIER SYSTEMS

Preformulation Studies:

FT-IR characterization:

The drug and excipient compatibility were assessed by FTIR Spectrophotometer. The need to characterize this is to explore the various functional groups of guest and host molecules by

examining significant changes in the shape and position of absorbance bands. The potassium bromide pellets were prepared by grinding 99% KBr with 1% of samples such as quetiapine fumarate, curcumin, cholesterol, oleic acid vitamin E TPGS, physical mixture of drug and excipients. The mixture was pressed in to transparent pellet using hydraulic press [17]

FTIR spectra obtained through the compatibility of the pure drug and excipient was observed using Bruker FTIR

DIFFERENTIAL SCANNING CALORIMETRY:

The physical characteristics of the NLC can be studied based on the thermograms obtained. This is done to evaluate any change in drug with respect to enthalpy , glass transition temperature and any interaction with excipients. Nearly 8 mg of sample was placed on aluminium pan and heated to the temperature of 50 -400C under nitrogen 40.0ml/min / N2, 60.0ml/min The instrument used was NETZSCH DSC 214 Polyma DSC21400A-0470-L

Experimental design:

Preliminary experiments and literature search revealed the influence of lipids used in the formulation of NLC. To discover the best circumstances and to produce the NLC, experimental design was carried out by design of experiment research (DOE) so as to optimize the quantities of lipids, minimize the number of trials thereby consuming time. This QbD by Design Expert Software used was version 7.0.0; Stat-Ease Inc. Minneapolis, USA. A 2 factors 2 level face-Response was used. The independent variables chosen are solid lipid (X_1) and Liquid lipid (X_2) and the response variables are particle size (Y_1) and transparency (Y_2). The experimental design details are given in table No: 1 which shows variables and constraints used in formulation. This design was specifically selected for exploration of complete design space with reduced experimental runs, without aliasing interaction factors.

Based on preliminary experiments the range values for the lipids were selected. The values are as follows:

Solid lipid value: 70% (minimum) to 90%(maximum)

Liquid lipid value: 10% -30%

TABLE 1: Independent and dependent variables with set of constraints for DOE

VARIABLES	**UNIT**	**LEVELS**		
INDEPENDENT		**LOW**	**MEDIUM**	**HIGH**
SOLID LIPID	Mg	70	80	90
LIQUID LIPID	mg	10	20	30
DEPENDENT	-	Constraints		
PARTICLE SIZE	nm	MINIMIZE		
TRANSPARENCY	%	MAXIMIZE		

Optimization and model validation:

With 2 factors to be studied by CCD consisted of 13 experimental runs having 4 factorial,4 axial and 5 central replicate points. The selected independent variable, X_1 and X_2, were varied at low, medium and high to study their impact on response variables, Y_1 and Y_2. The 2 independent

variables and their levels were chosen based on data obtained from trial runs. The model suggested by the software was selected after considering the lack of fit test and p-value for the model (at $p<0.05$). 2nd order polynomial equations of the following type were generated by the software to assess the effect of independent variables on response variables Y_1 and Y_2. Based on Lack of fit test and model statistic data, a suitable model was selected. With maximum desirability factor, optimized batch was selected based on dependent variable constraint. This optimized formulation of NLC was selected for further studies. The experimental design generated is shown in table no 2

TABLE NO 2: EXPERIMENTAL DESIGN (QbD) GENERATED BY THE DOE SOFTWARE

Run	Variable 1	Variable 2	
	Total 1%		Total
	A:Cholesterol	B:Oleic acid	
1	65.86	20.00	85
2	90.00	30.00	120
3	90.00	10.00	100
4	80.00	20.00	100
5	80.00	20.00	100
6	70.00	30.00	100
7	80.00	20.00	100
8	70.00	10.00	80
9	80.00	20.00	100
10	94.14	20.00	114
11	80.00	34.14	114
12	80.00	5.86	85
13	80.00	20.00	100

Final Equation in Terms of Actual Factors:

Particle size = +1017.98907 -18.29164 * Cholesterol -17.99541 * Oleic acid +0.17000 * Cholesterol * Oleic acid +0.090625 * Cholesterol ^{2} +0.12313 * Oleic acid^2

Transparence = -98.80456 +3.92322 * Cholesterol +3.75983 * Oleic acid -0.035000 *

$$\text{Cholesterol} * \text{Oleic acid} - 0.020625 * \text{Cholesterol}^2 - 0.033125 * \text{Oleic acid}^2$$

Preparation of nanostructured lipid carrier of quetiapine fumarate and curcumin:

Formulations were done by hot homogenization method. [18]Weighed quantities of cholesterol (solid lipid), oleic acid (liquid lipid), vit E TPGS were heated above 50 C to melt the mixture and to form molten mass. Weighed quantities of drug quetiapine fumarate and curcumin were added into molten mass and stirred to get uniform mixture. In another beaker HPLC grade water was taken as aqueous phase. Both aqueous phase and lipid phase were maintained at same temperatures and aqueous phase was slowly added to lipid phase followed by stirring and homogenization in a homogenizer (Remi, Electronik, Vasai, India, RQT 127/A/D) at 12000 RPM for ten minutes. This resulted in formation of hot clear aqueous and transparent NLC formulations. The composition table is given in table No 3

TABLE 2 COMPOSITION OF NLC

Code	Cholesterol (%)	Oleic acid (%)	Vitamin E TPGS (%)	Quetiapine fumarate (%)	Curcumin (%)	Water
QC-NLC1	90	10	5	0.1%	0.1%	Quantity sufficient to 100 ml
QC-NLC2	80	20	5	0.1%	0.1%	
QC-NLC3	70	30	5	0.1%	0.1%	
B-NLC1	90	10	5	-	-	
B-NLC2	80	20	5	-	-	
B-NLC3	70	30	5	-	-	

METHOD DEVELOPMENT

Reverse phase HPLC method for quetiapine fumarate and curcumin:[19]

The complete chromatographic separation and estimation of both drugs was possible using phenomenex kinetex XB-C18 100 A analytical column (3.5 µm, 4.6 mm × 150 mm) and methanol: water 70:30 v/v as a mobile phase, at the flow rate of 1.0 mL/min. Sample of 200 µL was injected and chromatogram was observed.

Characterization of NLC

Particle Size and Zeta Potential

The average particle size and zeta potential of all the formulated QC NLC was determined by combination of phase analysis light scattering technique and, laser Doppler micro-electrophoresis using Zetasizer (Nano ZS, Malvern Instruments, UK). The analysis was performed

in triplicates and the particle size and zeta potential of QC-NLC was expressed as the value of average size . Zeta potential reflects the electric charge on the particle surface and physical stability of colloidal systems. The homogeneity of the particle size distribution was indicated by the polydispersity index (PDI). Polydispersity index indicates the distribution of particle size of nanoparticles which reveal nature of distribution like monodisperse and polydisperse.[20]

Cloud point Determination

Cloud point is the temperature above which an aqueous solution of a water-soluble surfactant becomes turbid. Determination of cloud point is important to know the storage stability of the surfactant incorporated formulations. Cloud points are characteristic of nonionic surfactants. The formulations were placed in a water bath and the temperature was gradually increased. The decreases in transparency were due to the formation of cloudiness in the QC-NLC were observed visually and the temperature at which the cloudiness occurs was noted as cloud point.[21]

Transparency determination:

The transparency of the formulated QC-NLC was evaluated at 800 nm by double beam UV-Visible Spectrophotometer (UV-1700, Shimadzu, Japan) using distilled water as a blank (Song et al 2010).[22]

TRANSMISSION ELECTRON MICROSCOPY (TEM)

The shape and morphology of the NLC formulation was characterized by transmission electron microscopy (TEM, TOPCON 002B) with an accelerating voltage of 200 kV. On a carbon-coated 200-mesh copper grid a drop of the NLC dispersion was placed to create a thin film. It was negatively stained with 1.3% (w/v) phosphotungstic acid by adding a drop of the staining solution to the film. The film was dried on the grid, for 30 s; any excess droplets were drained off using filter paper. The grid was allowed to air-dry under room temperature and samples were observed by TEM.[23]

ATOMIC FORCE MICROSCOPY:

The atomic force microscopic study on the formulated NLC is bring to the light the external morphology possessed by the NLC's. The formulation was diluted with distilled water and using a drop that was dried as film on the slide the study was carried out using multimode scanning probe microscope of the model AFM/STM (NT-MDT) [9]

Determination of Drug content

Drug content of all the formulated QC NLC was evaluated by transferring QC NLC (1ml) into a 10 mL volumetric flask containing 4 ml of methanol. It was then sonicated for 15 minutes and diluted using methanol and filtered through 0.22 µm membrane filter. Aliquot of the filtrate was further diluted with pH 1.2 buffer and evaluated by RP-HPLC method. The chromatographic separation was achieved using phenomenexkinetex XB-C18 100 A (3.5 µm, 4.6 mm × 150 mm) analytical column and methanol : water (70:30 v/v) as a mobile phase, at the flow rate of 1.0 mL/min. Sample of 200 µl was injected and chromatogram was recorded at 290 nm in triplicates.

Determination of Entrapment Efficiency (% EE)[24]

The % entrapment efficiency of QC-NLC was evaluated by direct method. The QC-NLC was centrifugation at 12000 rpm for 30min in a cooling centrifuge at 4 °C (C-24, Remi). The NLC were solubilized in methanol and filtered through 0.22 µm membrane filter. Aliquot of the filtrate was further diluted with pH 1.2 buffer and evaluated by RP-HPLC method. The Entrapment efficiency (EE) was calculated by the following equation:

$$EE(\%) = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

***IN-VITRO* DRUG RELEASE**

In-vitro drug release of all the NLC formulations were evaluated using a dialysis bag (cut off 5000 Da, Himedia, India) method. The drug loaded NLC (1 ml) was placed in dialysis bag, hermetically sealed and suspended in 50 ml of dialyzing medium. The temperature was maintained at 37±0.1°C using closed double jacketed thermostatic chamber and stirred at 600 RPM using magnetic stirrer. Phosphate buffer pH 7.4 with methanol (50:50) was used as a dialyzing medium for QC-NLC.

Aliquots of 2 ml samples were withdrawn at predetermined intervals and the receptor compartment was replenished with same volume of the fresh dialyzing medium. The aliquots were diluted suitably with buffer pH1.2 and analyzed in triplicate by RPHPLC system using method described earlier. The results were expressed as average cumulative percentage drug released versus time.

STABILITY STUDY:

QC-NLC formulations were kept in stability chamber. The stability study of the nanodispersion was carried out based on ICH guidelines by storing the samples at 40±2°C/ 75±5% RH for 180 days in the stability chamber [25]

EVALUATION OF PREPARED NLC BY INSILICO METHODS: [26]

With the advent of developing various software tools are being developed as a part of computer-aided structure-based drug design, virtual screening, and in silico pharmacokinetics predictions. Identification of novel inhibitors of dopamine transporters (DAT) with better therapeutic potential for the treatment of schizophrenia-related cognitive impairments and other neuropsychiatric disorders. Structure base drug design approach has been widely employed in analysis of molecular recognition through binding affinities, molecular interactions, induced conformational changes, and toxicity filters for the design and identification of novel compounds.

Target selection and retrieval

The three-dimensional structures of the target proteins including Dopamine transporter (DAT) (PDB ID:4m48); D2 Dopamine receptor (PDB ID: 6cm4) and D3 Dopamine receptor

(PDB ID: 3pbl), and GLUT1 (PDB ID: 4pyp) were retrieved from the PDB (Protein Data Bank).[27] The retrieved proteins were checked for missing residues. The selected proteins, D3 Dopamine receptor, D2 receptor, GLUT1 and SLC1A2 were checked for missing residues.

Homology Modelling

Since the D3 dopamine receptor had missing residues in its respective chain and the 3D structure of SLC1A2 was not available, they were subjected to homology modelling using the SWISS-MODEL Server. For the modelling of D3R and SLC1A2 the target sequences were aligned with the template sequences and the respective models were generated.

Using Ramachandran plots of both D3 Receptor and SLC1A2 showed that about 95.2% and 87.9% residues were present in the favoured regions exhibiting that the models were feasible.

Ligand retrieval

The three-dimensional structures of the selected ligands including Curcumin (PubChem CID: 969516), Quetiapine (PubChem CID: 5002), Vitamin E TPGS (PubChem CID: 71406), Cholesterol (PubChem CID: 5997) and Oleic acid (PubChem CID: 445639) were retrieved from the PubChem database

ADME and Toxicity [28-31]

Using the Swiss ADME server, ADMET LaB 2.0 was used to evaluate the blood-brain permeation (BBB), substrate for permeability glycoprotein (P-gp). Toxicity parameters of the ligands were examined using ProTox-II online tool . Molecular weight and Log P values are determined by Molinspiration tool.

Molecular Docking [32]

Molecular docking between the proteins and ligands were performed using Auto Dock 4.2 – Auto Dock Tools 1.5.6. Grid with a spacing of 0.436 Å with the 40 × 40 × 40 dimensions was generated around the active sites of target proteins. Native ligand-bound sites and the active sites identified using CAST p 3.0 server was used as active sites. Finally, the grid parameter (gpf) was generated. Then, the docking parameter file (dpf) was generated using the Lamarckian algorithm and search parameters were set to genetic algorithm. Molecular docking analysis was performed by the run module using "autogrid4.exe" and "autodock4.exe". Then, the molecular interaction between the protein and ligand complexes was assessed using UCSF Chimera and LigPlot+ visualization tool.[33]

RESULTS AND DISCUSSIONS

Preformulation Studies:

FTIR:

The IR spectra of drug, excipients and physical mixture were studied and analysed for interaction between drug and excipients using FTIR specification. The spectra obtained showed characteristic peaks of components which proved the identity and authenticity of the drug and excipients. The FTIR spectra of physical mixture showed no additional peaks other than the characteristic peaks proving the compatibility between drug and excipients. The FTIR spectra is shown in figure no 1

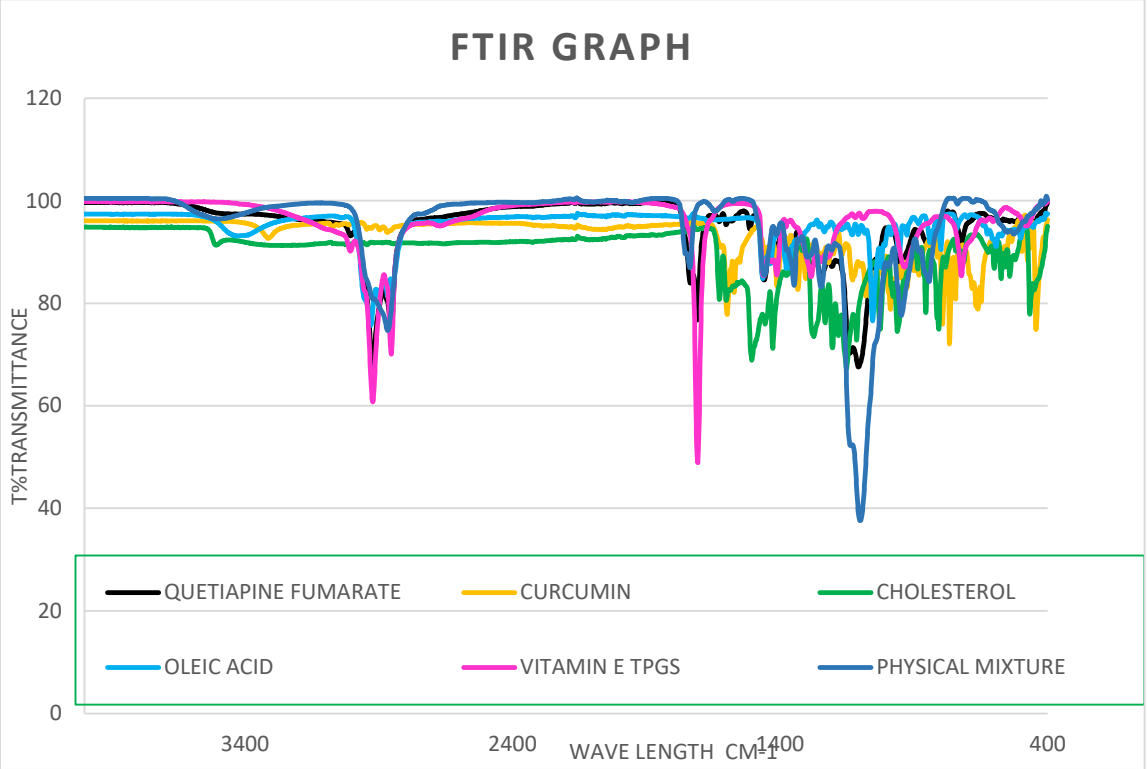


FIGURE N0 1: FTIR SPECTRA OF QUETIAPINE FUMARATE, CURCUMIN, CHOLESTEROL, OLEIC ACID, VITAMIN E TPGS AND THE PHYSICAL MIXTURE

Differential scanning calorimetry:

The endothermic peak is characteristics of each compound. A sharp endothermic peak of a compound represents its melting point. It is a powerful tool to analyse the crystallization and interaction between the excipients by the determination of variation of temperature and energy phase transition. The thermogram showed a sharp endothermic peak at 173.64C for quetiapine fumarate representing its melting point and its characteristic crystallinity.

At the same time thermogram for curcumin the peak was observed at 174.86 C that specify its characteristics. In case of cholesterol, it was at 149.32C. These specified characteristic peaks were absent in the formulation indicating no interaction between the excipients and also indicates that drugs were perfectly blended in amorphous form and encapsulated within lipid matrix

providing holistic picture of genuine encapsulation. The thermograms are shown below in figure no 2

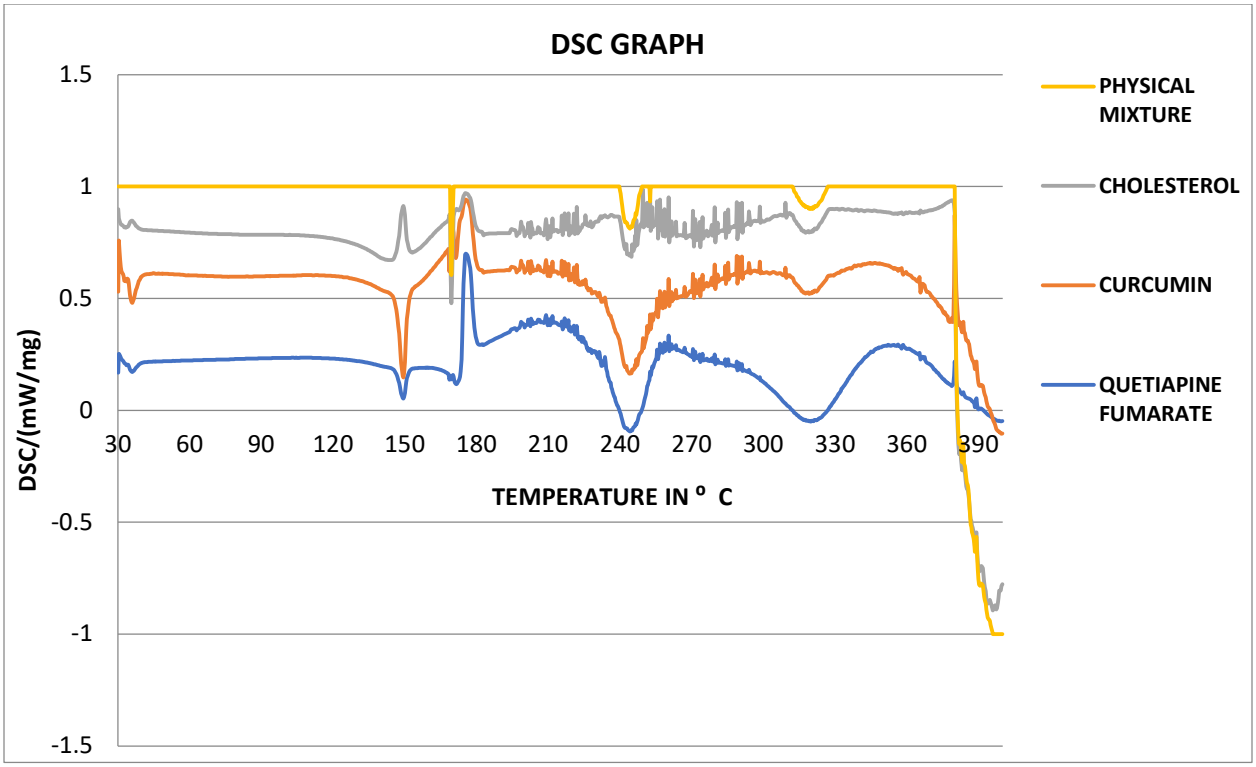


FIGURE NO: 2 DSC OF QUETIAPINE FUMARATE, CURCUMIN, CHOLESTEROL AND PHYSICAL MIXTURE

Optimization of design of experiments:

The optimization helped us to optimize the lipids used to formulate NLC with minimum number of test runs. The variables optimized are particle size and transparency. The table 3 shows the results of experimental runs for the two factors transparency and particle size. The particle size ranged from 94.14 to 218.3 nm and transparency was 70.79 to 90%. The mathematical relationship was established using Design- Expert software (DoE) (version 7.0.0; Stat-Ease Inc. Minneapolis, USA).

TABLE NO 3 RESULTS OF EXPERIMENTAL DESIGN

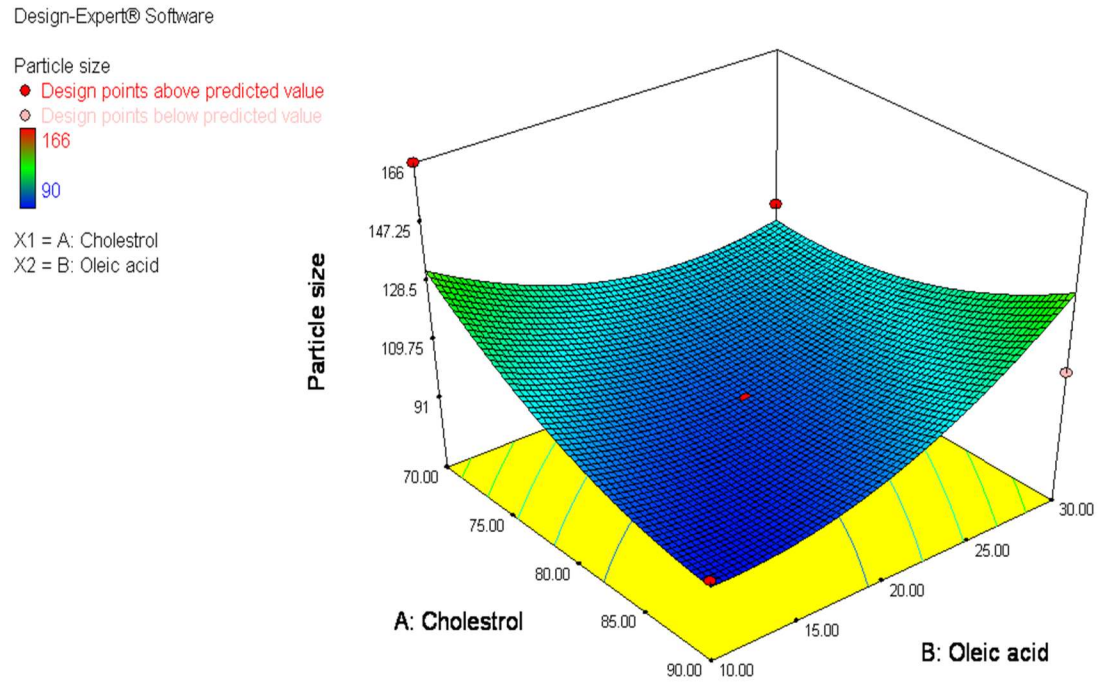
Run	Variable 1	Variable 2	Total	Response variables	
	Total 1%			Particle size nm	Transparence %
	A:Cholesterol	B:Oleic acid			
1	65.86	20.00	85	94.14	85.96
2	90.00	30.00	120	132.1	75.71
3	90.00	10.00	100	104.7	90.01

4	80.00	20.00	100	96.00	89.00
5	80.00	20.00	100	96.00	89.00
6	70.00	30.00	100	144.5	84.24
7	80.00	20.00	100	96.00	89.00
8	70.00	10.00	80	166.2	74.54
9	80.00	20.00	100	96.00	89.00
10	94.14	20.00	114	218.3	70.79
11	80.00	34.14	114	172.2	77.21
12	80.00	5.86	85	113.13	87.54
13	80.00	20.00	100	96.00	89.00

Particle size =+1017.98907 -18.29164 * Cholesterol -17.99541 * Oleic acid +0.17000 * Cholesterol * Oleic acid +0.090625 * Cholesterol ^{2} +0.12313 * Oleic acid^2

Transparency = -98.80456 +3.92322 * Cholesterol +3.75983 * Oleic acid -0.035000 * Cholesterol * Oleic acid -0.020625 * Cholesterol^2 -0.033125 * Oleic acid^2

The 3D response surface graphs were given in figure 1a and Figure 1b



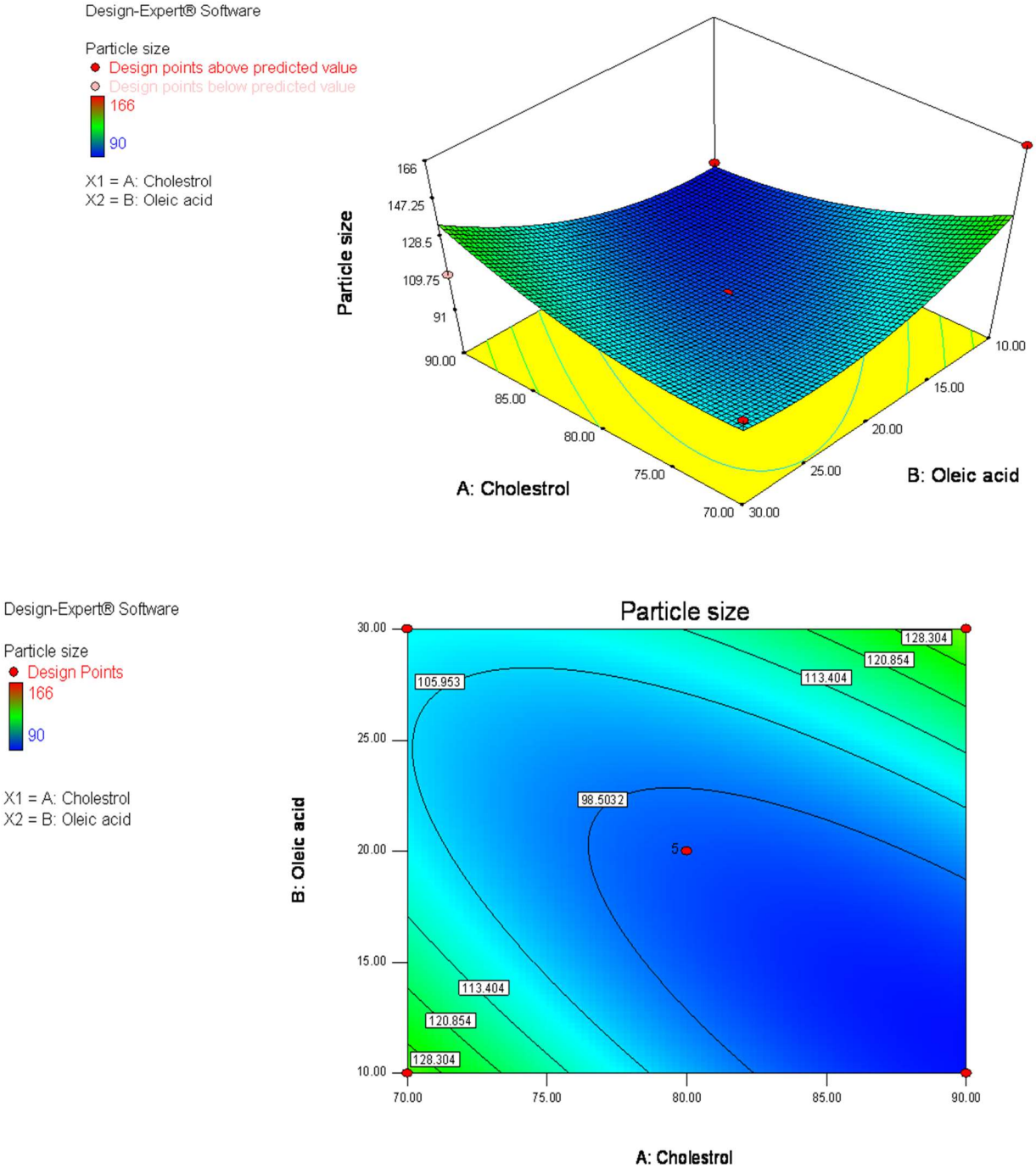
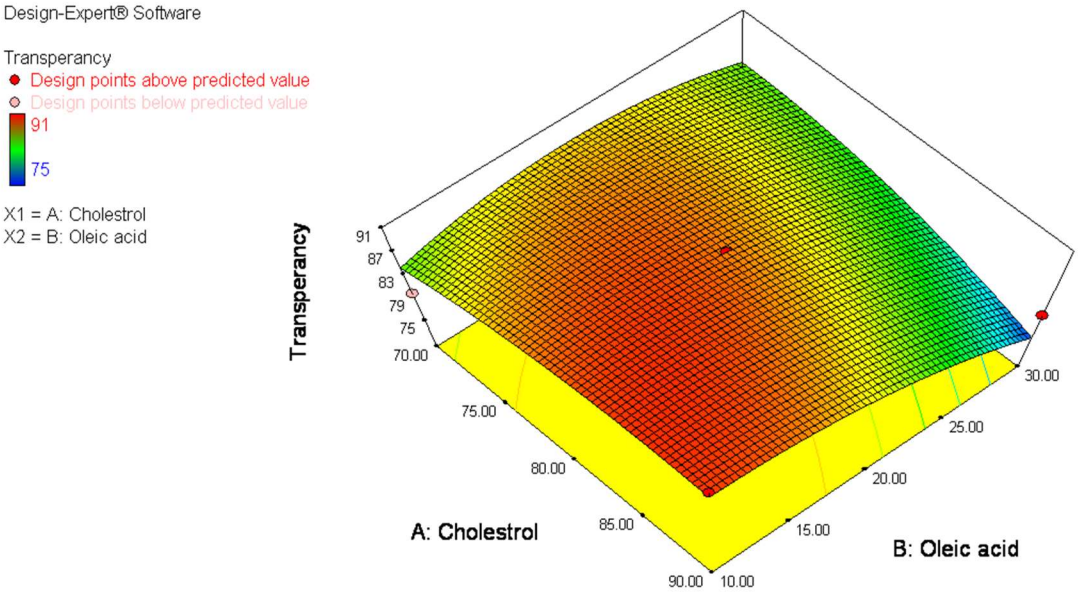
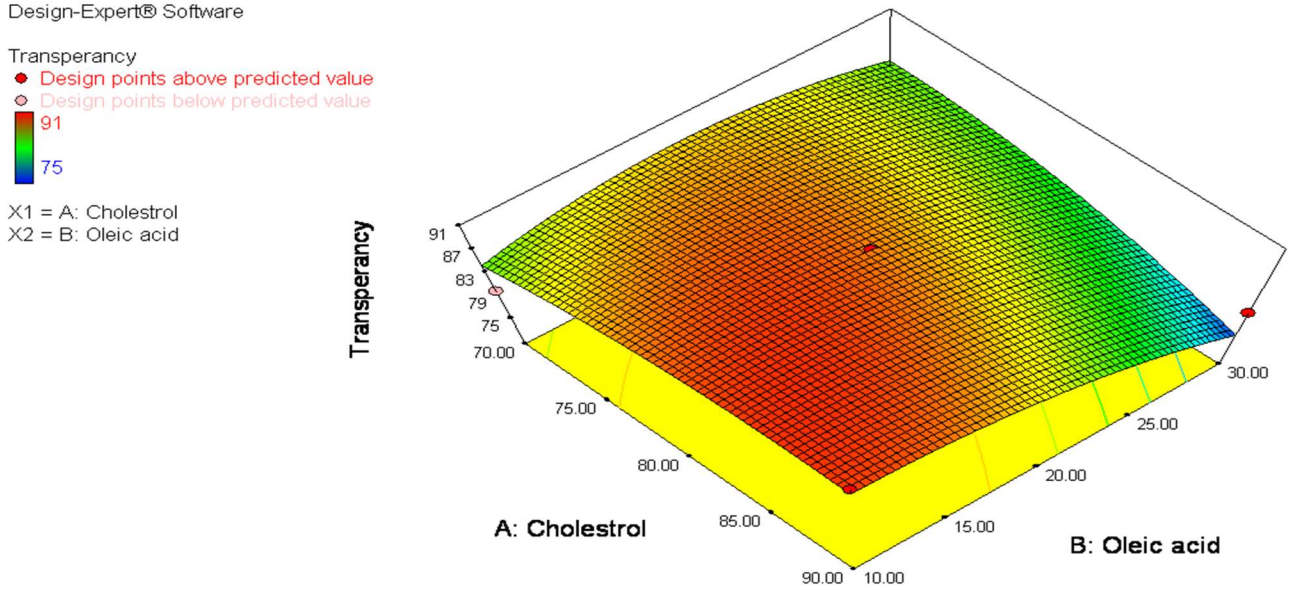


FIGURE 3A: 3D AND 2D RESPONSE SURFACE PLOTS MANIFESTING THE EFFECT ON INDEPENDENT VARIABLE SOLID LIPID AND LIQUID LIPID ON PARTICLE SIZE


Design-Expert® Software
Transperancy
Design points above predicted value
Design points below predicted value
91
75
X1 = A: Cholestrol
X2 = B: Oleic acid
Transperancy
91
87
83
79
75
70.00
75.00
80.00
85.00
90.00
A: Cholestrol
10.00
15.00
20.00
25.00
30.00
B: Oleic acid


Design-Expert® Software
Transperancy
Design points above predicted value
Design points below predicted value
91
75
X1 = A: Cholestrol
X2 = B: Oleic acid
Transperancy
91
87
83
79
75
70.00
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15.00
20.00
25.00
30.00
B: Oleic acid

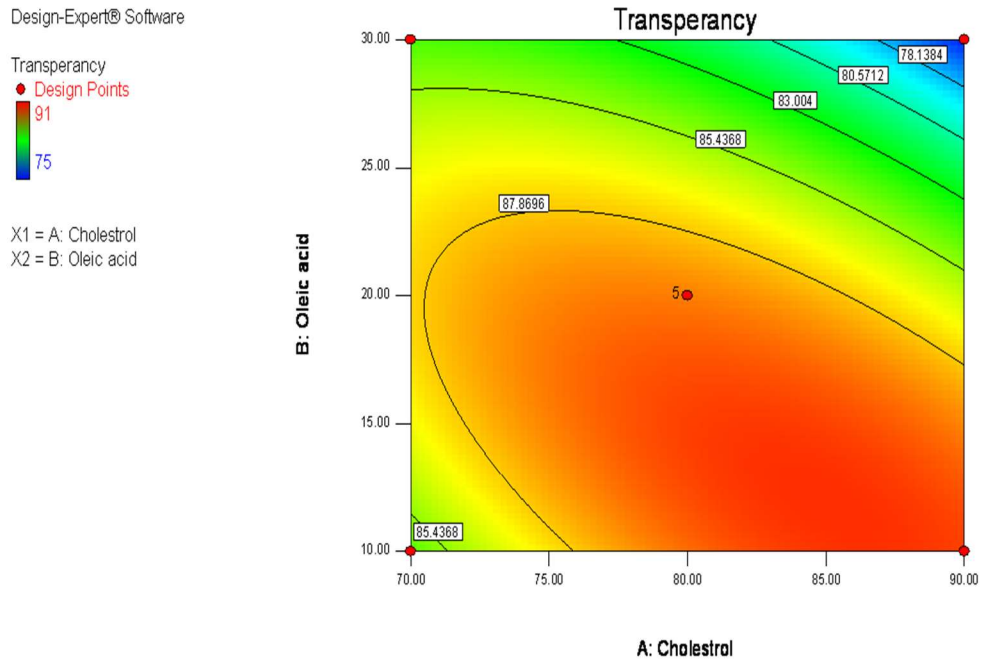

FIGURE 3 B : 3D AND 2D RESPONSE SURFACE PLOTS MANIFESTING THE EFFECT ON INDEPENDENT VARIABLE SOLID LIPID AND LIQUID LIPID ON TRANSPARENCY

From the graphs it is seen that increase in cholesterol ie solid lipid, there is decreases the particle size of the formulation. In case of oleic acid, the liquid lipid the increase in concentration oleic acid, decrease in particle size up to 25% and then slight increase in particle size is observed.. Similarly with increase in concentration of solid lipid cholesterol the transparency increased and it decreased with increase in oleic acid concentration

In 3D RSM (3D and Contour plot (Fig.3) blue colour indicates the formation smallest of particle size and red colour indicates the maximum transparency of the formulations. The presence of major region of blue colour and small region of green colour indicates the smaller particle sizes of the developed formulations.

The increase in red colour of graph with decrease in concentration of oleic acid and their contribution towards the transparency of the formulations. RSM diagrams thus showed that the higher the concentration of solid lipid cholesterol and lower concentration of liquid lipid produces the lower particle size and higher transparency of NLC formulations.

Formulation of QC NLC

Based on results of experimental designs the quantities of lipids were fixed and formulations were made. The compositions were given in table no 4

TABLE NO 4: COMPOSITION OF NLC

Code	Cholesterol (%)	Oleic acid (%)	Vitamin E TPGS (%)	Quetiapine fumarate (%)	Curcumin (%)	Water	Particle size(nm)	Transparency %
QC-NLC 1	90	10	5	0.1% (10 mg)	0.1% (10 mg)	Qs to 100 mL	93	91
QC-NLC 2	80	20	5	0.1% (10 mg)	0.1% (10 mg)	Qs to 100 mL	96	89
QC-NLC 3	70	30	5	0.1% (10 mg)	0.1% (10 mg)	Qs to 100 mL	115	83
B-NLC 1	90	10	5	-	-	Qs to 100 mL	90	88
B-NLC 2	80	20	5	-	-	Qs to 100 mL	91	85
B-NLC 3	70	30	5	-	-	Qs to 100 mL	96	83

CHARACTERIZAION:

PARTICE SIZE, POLYDISPERSITY INDEX AND ZETAPOTENTIAL

Malvern equipment was used to determine particle size and zetapotential .The observed results are shown in table no 5.

TABLE NO 5 OBSERVED VALUES OF PARTICLE SIZE, ZETA POTENTIAL AND POLY DISPERSITY INDEX

FORMULATION CODE	PARTICLE SIZE (nm)	ZETA POTENTIAL (Mv)	POLYDISPERSITY INDEX
F1	94.14 ±0.9	-20.8± 1.2	0.172± 0.11
F2	110.2±0.4	-13.9 ± 1.8	0.158± 0.01
F3	93.8±0.3	-9.34± 1.4	0.099± 0.21

F4	96.02±.01	-7.76± 1.1	0.112± 0.13
F5	115.7±0.7	-5.70± 1.0	0.245± 0.12
F6	166.2±0.11	-6.19± 0.9	0.258± 0.16
F7	127.4±0.2	-3.68± 0.7	0.229± 0.11
F8	144.4 ±0.6	-4.60± 2.2	0.33± 0.31

Data are mean ± SD , n=3

Transmission electron microscopy:

To determine the size of nanostructured lipid particulate system, the preparation was focused using transmission electron microscope. The photos were taken and the NLC particles were observed to be spherical with curved surface. The TEM pictures were given figure 4

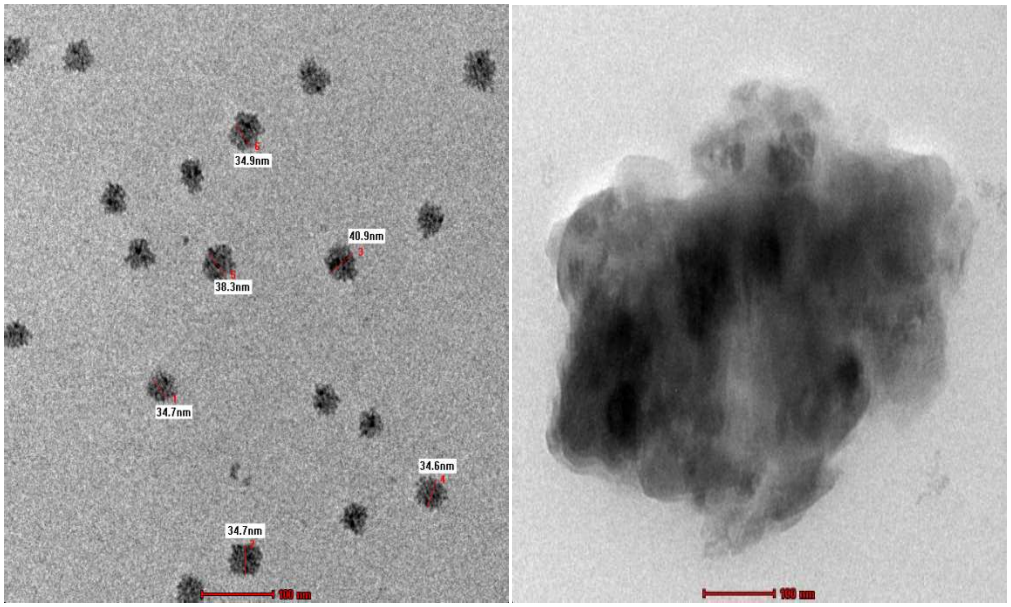

FIGURE 4: SHOWING PARTICLE MORPHOLOGY OF NLC BY TRANSMISSION ELECTRON MICROSCOPY

Cloud point:

The temperature at which clarity of formulation turns is called cloud point. The cloudiness appeared in the tested formulations were gets disappeared when the temperature decreases just below the cloud point and it happens within few minutes. The cloud point was in the range 74^0C-77^0C

Drug Content and entrapment efficiency:

The drug content percentage of curcumin and quetiapine fumarate were above 98%. The entrapment efficiency which shows the encapsulation of drug within NLC matrix was 98.28, 98.90,97.99% for quetiapine in NLC 1, NLC 2 and NLC 3 formulations respectively. The curcumin concentration was above 97% in all formulations. The results were shown in table no:6

TABLE 6: DRUG CONTENT, ENTRAPMENT EFFICIENCY AND CLOUD POINT OF THE FORMULATED QC-NLC FORMULATIONS

Formulation Code	Drug content (%)		Entrapment efficiency (%)		Cloud point (^{0}C)
	QP	CUR	QP	CUR	
QC-NLC1	99.80	98.63	98.28	97.8	77
QC-NLC2	99.38	98.10	98.90	97.42	76
QC-NLC3	99.08	98.05	97.99	98.21	74

ATOMIC FORCE MICROSCOPY:

The surface characterization was shown by AFM. The morphology of the particle and the particle size was observed to be small. The pictures are depicted in figure no 5

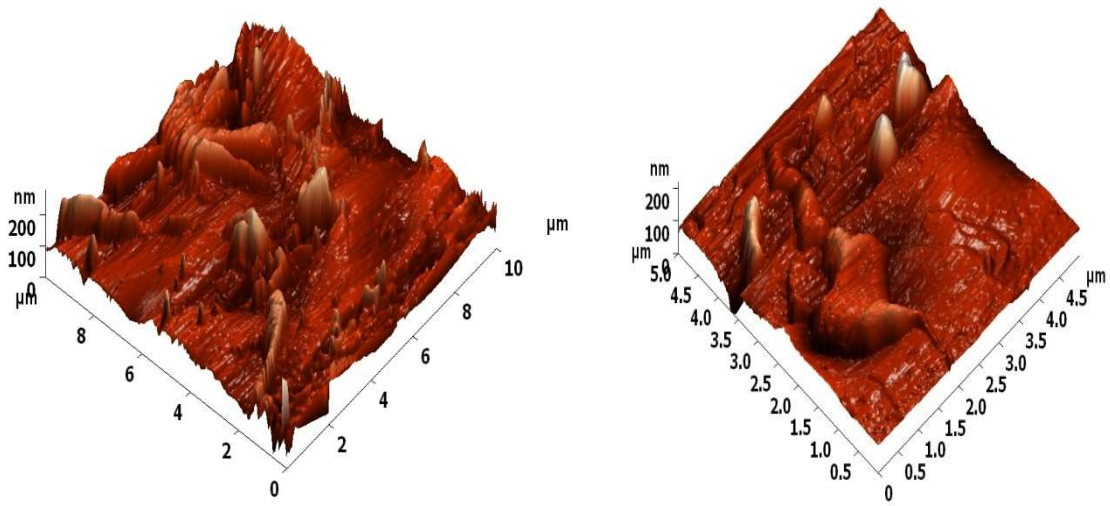

FIGURE NO : 5 AFM IMAGE OF QC-NLC (10µm X 10 µm X 200 nm)

Invitro release studies of QC –NLC:

The invitro drug release studies showed that drugs are perfectly entrapped in lipid matrix. Among the three formulations (QC-NLC 1, QC-NLC 2 and QC-NLC 3), the drug release in case of QC-NLC 3 formulation which contains 70% cholesterol and 30% oleic acid. The release of quetiapine fumarate was 73.18% and for curcumin the release of drug was 42.18% from the nano structured lipid carrier formulations at the end of 72 hours. In case of QC-NLC 1 formulation (F1- in this) the release of quetiapine fumarate and curcumin were 90.73% and 63.59% respectively. This QC- NLC 1 formulation had 90% cholesterol and 10% oleic acid. The percentage drug release for the formulation containing 80% cholesterol and 20% oleic acid, the release rate was 51.35% and 74.51 %for curcumin and quetiapine fumarate respectively (F2 in graph). The release rate of

these formulations were compared with that of pure quetipaine fumarate and pure curcumin also which showed 20.84% and 21% indicating very slow release at the end of 72 hours. The results are shown in graph ,figure no 6

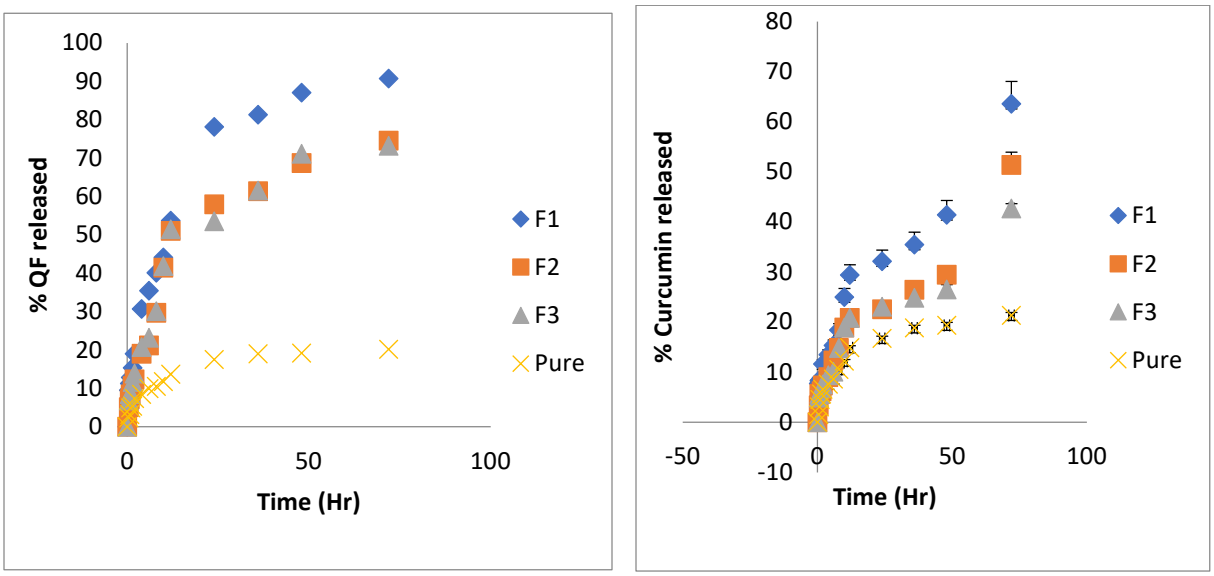

FIGURE NO 6: GRAPHS SHOWING INVITRO RELEASE PROFILES OF QUETIAPINE FUMARATE AND CURCUMIN

Stability studies:

The NLC formulations showed good stability. Among the formulations QC-NLC 1 showed much more stablity in all parameters compared to the other two formulations. All the formulations were physically stable with no change in physical appearance, or phase separation. The particle size ,transparence ,zeta potential, PDI, % entrapment efficiency and other parameters were maintained after 6 months during stability study at 40±2°C/ 75±5% RH for 180 days in the stability chamber.

EVALUATION OF QC-NLC BY INSILCO MOLECULAR DOCKING ANALYSIS:

ADME

Total polar surface area (TPSA Å²) and Log S are the ideal parameters to be evaluated, which influences the solubility and bioavailability of the molecule should be < 160 Å² and in the range of -4.0 to 0.5 log mol/L.

Log P and Molecular weight was determined by Molinspiration tool. Except vitamin E TPGS all the ligands have a MW < 500. Log P is a crucial parameter that should be less than 5, whereas Curcumin and Quetiapine showed log P values less than 5.

A drug-like molecule's efficacy can be gauged by testing it against the ADMET properties, and in cases where toxicity is a crucial factor in computational models used to predict compounds' hazards, this is an absolute. Quetiapine and cholesterol shown to have immunotoxic effects,

negative for all the other type of toxicities. The results of ADME and Toxicity are shown in table no 7

TABLE NO : 7 ADME ANALYSIS

Ligands	**ADME**				**Drug likeness by** Molinspiration tool	
	TPSA Å²	**Log S**	**BBB**	**P-gp**	**Mol.wt (daltons)**	**Log P**
Curcumin	93.07	**-3.92**	-	No	368.38	2.30
Quetiapine	48.83	-3.50	+	Yes	383.52	3.49
Vitamin E TPGS	82.07	-8.42	-	No	574.84	8.57
Cholesterol	20.23	-7.40	+	No	386.66	7.62
Oleic acid	37.30	-5.41	-	Yes	282.47	7.58

TPSA-Topological Polar Surface Area **Log S** – Solubility (-4~0.5 log mol/L); **hERG** – hERG potassium ion channel inhibitor (- ~ non-toxic ; + ~ toxic) ; **BBB –** Blood brain permeation; **P-gp** - substrate for permeability glycoprotein - ("+" – Substrate: "-" – not a substrate)

TABLE 8 TOXICITY PREDICTION ANALYSIS

	Toxicity prediction				
Ligands	**HT**	**CG**	**IT**	**MG**	**CT**
Curcumin	Inactive	Inactive	Inactive	Inactive	Inactive
Quetiapine	Inactive	Inactive	active	Inactive	Inactive
Vitamin E TPGS	Inactive	Inactive	Inactive	Inactive	Inactive
Cholesterol	Inactive	Inactive	active	Inactive	Inactive
Oleic acid	Inactive	Inactive	Inactive	Inactive	Inactive

HT-Hepatotoxicity; **CG** – carcinogenicity**; IT** – immunotoxicity; **MG** – mutagenicity; **CT** – cytotoxicity;

Molecular docking Analysis

Table 9. Molecular docking analysis

Ligands	**DAT**	**D2R**	**D3R**	**GLUT1**	**SLC1A2**
	Binding energy (Kcal/mol)				
Curcumin	**-8.18**	**-5.76**	**-5.55**	**-5.51**	**-7.37**
Quetiapine	**-9.24**	**-5.52**	**-5.26**	**-6.55**	**-8.42**
Vitamin E TPGS	>0	>0	-3.52	>0	-4.27
Cholesterol	**-8.41**	>0	**-8.45**	**-5.75**	**-9.60**

Oleic acid	-4.72	-4.83	**-6.21**	-3.33	**-6.03**

Dopamine transporter (DAT):D2 Dopamine receptor (D2R) and D3 Dopamine receptor (D3R): Glucose transporter 1(GLUT1): Excitatory amino acid transporter 2/ Excitatory amino acid transporter 2 (SLC1A2).

DAT, D2R, D3R, GLUT1 and SLC1A2 against the ligands (Curcumin, Quetiapine, Vitamin E TPGS, Cholesterol and Oleic acid). Thus, the ligand complexes showing the lowest binding energy below -5.0 Kcal/mol were considered significant. Thus, the curcumin and quetiapine has shown to have significant binding affinity towards all the targets. While cholesterol have shown to have binding affinity towards all the targets except D2R and oleic acid only shown affinity towards D3R and SLC1A2.

Dopamine transporter (DAT) is a monoamine transporter that plays a role in the regulation of dopamine (DA) neurotransmission as well as other physiological processes and neurological and psychiatric diseases. DAT is a building block membrane protein that has the function of removing dopamine from the synaptic cleft and accumulating it in the cells that are surrounding the cleft. This results in the termination of the signal that the neurotransmitter was transmitting.

A clinical illness described by disordered mind that is generally known as schizophrenia can be caused by an excessive amount of dopamine neurotransmission.[35] Thus, DAT is a viable target for treating schizophrenia. Curcumin, quetiapine and cholesterol has shown to have affinity towards DAT with binding energy lower than -5.0 Kcal/mol. These are depicted in figure no 7 and 8.

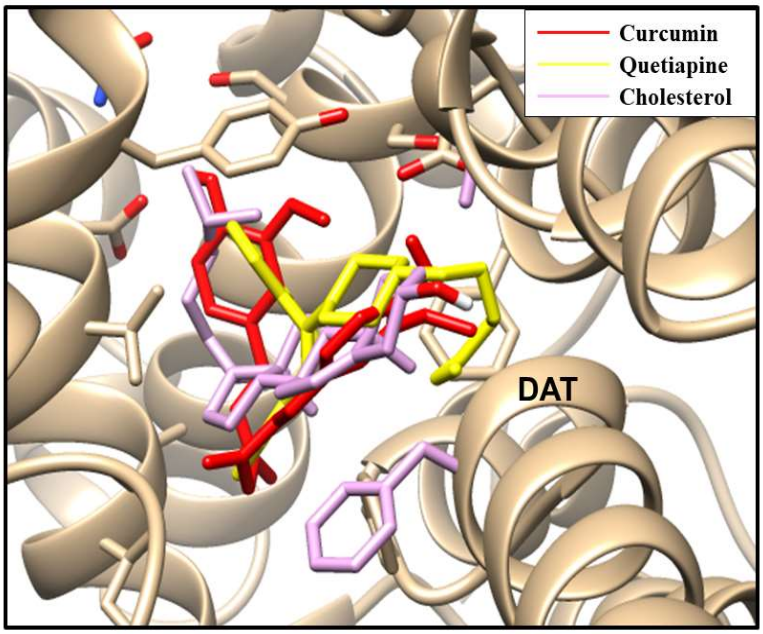

FIGURE NO 7: 3D DOCKING POSE OF CURCUMIN, QUETIAPINE AND CHOLESTEROL AGAINST DAT.

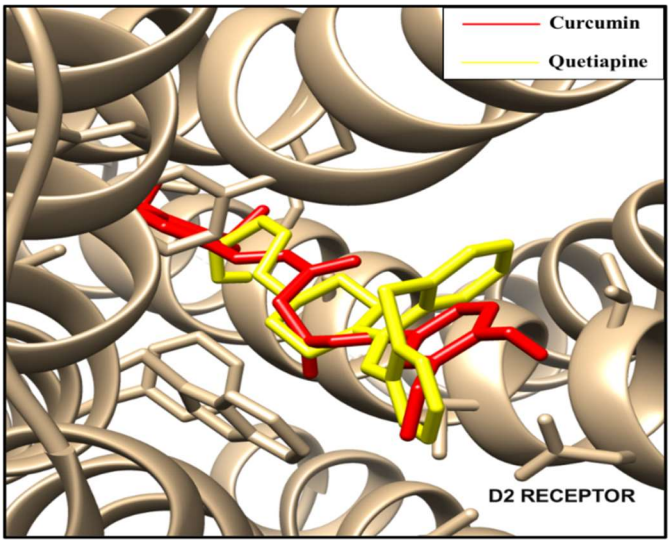

FIGURE NO 8 3D DOCKING POSE OF CURCUMIN AND QUETIAPINE AGAINST D2 RECEPTOR.

Curcumin has shown to form two conventional H-bonds with the amino acid residues ASP121 (3.01Å) and ASP46 (2.74 Å) and three π-σ bonds with the amino acid residues PHE43, VAL120 and TYR 124 (Fig 9a). Quetiapine formed a conventional H-bonds with the PHE319 (2.61 Å) and a π-σ bond with the residue VAL120 along with two π-alkyl bonds to the ALA117 and TYR124 (Fig 9b.).

Cholesterol has formed a conventional H-bond with PHE319, a π-σ bond with TYR124 and eight π-alkyl with the residues including PHE43, ALA48, ALA117, VAL120, PHE325 and ALA479 (Fig 9c.).

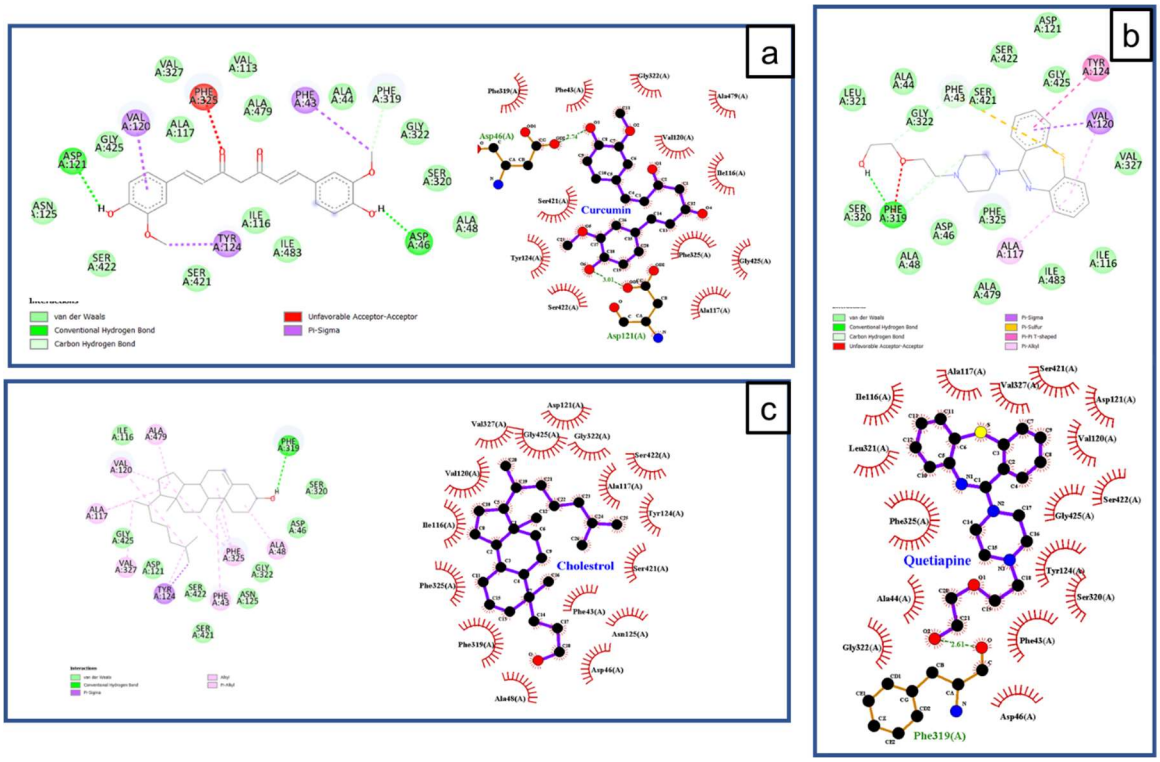

FIG 9. 2D MOLECULAR INTERACTION BETWEEN DAT-LIGAND COMPLEXES.

A) CURCUMIN B) QUETIAPINE C) CHOLESTEROL.

In case of D2R, curcumin and quetiapine showed considerable binding affinity with free binding energies of -5.76 and -5.52 Kcal/mol respectively. Curcumin has conventional hydrogen bonds with ASP114, PRO201, PHE202 and TRP386 residues, π-alkyl bonds with VAL115, CYS118, ALA122, CYS126 residues of D2 dopaminergic receptors. Quetiapine has formed an H-bond with ALA122 (3.05Å) and π-alkyl bonds with the two amino acid residues VAL115 and CYS118.

Schizophrenia patients are supersensitive to dopamine-like drugs like amphetamine or methylphenidate, meaning they respond with increased psychotic symptoms. Super sensitivity may be caused by an increase in pre-synaptic dopamine release or post-synaptic D2 or D2High receptors in active schizophrenia. When compared to neuropsychiatric healthy control subjects, schizophrenia patients had higher levels of D2 mRNA in the frontal cortex [36-37]. It has been discovered that schizophrenia patients have a significantly increased density of D (2)-receptors in their striatum, in addition to having a slightly increased density of D (2)-receptors in their basal conditions.[38] Thus, D2 receptor is a potential target for the treatment of schizophrenia

Conclusion:

In this study an attempt is done to formulate nano structured lipid carrier (NLC) using quetiapine fumarate in the treatment of schizophrenia. Being substrate at BBB their entry is facilitated by adding curcumin. Hot homogenization was followed and by sonication NLC's were

prepared. The number of trials and the experiment was designed by DOE .2^2 factorial designs were followed with solid lipid and liquid lipid as variables and particle size and transparency as response. The particle size as found to around 93 nm with transparence around 90%. The TEM and AFM revealed the morphology of the beads. The invitro release studies showed that quetiapine fumarate and curcumin were released in sustained form for QC-NLC 3 with the release of 73.18% for curcumin and 42.18% for quetiapine fumarate. The formulation QC-NLC 1 was found to be more stable ,the particle size, release rate , entrapment efficiency were more satisfactory and considered as ideal formulation.

The presence of vitamin E TPGS, surfactant helps in maintaining stability of the formulation , the formulations with respect to particle size , morphology and entrapment efficiency holds good after 6 months of storage at 40±2°C/ 75±5% RH for 180 days in the stability chamber.¨ Solid state characterization revealed the compatibility of drug and excipients and crystallinity of the drug is maintained in the formulation. The assessment of BBB targeting is accomplished by in silico studies, ADMET analysis disclosed that this NLC formulation could be administered intranasally for blood barrier targeting.

Further the molecular docking analysis proved that, binding of the formulation to D2 and DAT receptors thereby reducing the flaws related to drug's bioavailability, targeting the site with reduced dosing frequency and side effects. Hence NLC of quetiapine fumarate and curcumin are best combination in achieving desired therapy for schizophrenic patients. The formulation could be administered intranasally to achieve the desired effect.

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