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### RESEARCH ARTICLE

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## SYNTHESIS AND BIOPHARMACEUTICAL EVALUATION OF NANOPARTICLES CONTAINING RESVERATROL, AN ANTI CANCER DRUG

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#### Abstract

The chemotherapy of cancer disease become increasingly important in recent years because ease of operation. The traditional cancer chemotherapy is based on the promise that tumor cells are more likely to be killed by anticancer drugs because of the faster proliferation of those cancer cells. However, in reality most of the drugs cannot differentiate cancer cells from the normal cells. This result the undesirable effect of the drug because of the lack of selectivity i.e the patients always at risk of cytotoxicity. So the development of tumor targeted drug delivery systems in which they recognize the intrinsic difference between normal cells and tumor cells is an important requirement for effective tumor therapy. These carriers include nanoparticles, nanotubes, Nano rods, centromeres, micelles, solid lipid nanoparticles, microspheres. (1) In this experiment, all the materials used in the experiments were of analytical grade. Synthesis of CDI-activated poloxamer-407, NH<sub>2</sub> terminated poloxamer-407, Folate conjugated poloxamer-407 and prototype formulation of Liquid crystalline nanoparticle were prepared. Drug loading was done with the optimized formula. In vitro drug release from the nanoparticles was determined in phosphate buffer pH 7.4 and Zero-order model, first order model, Higuchi's model, kosmeyer-Peppas model also tried for compatibility. The cytotoxicity studies in the final formula by Brine shrimp experiments were done and found that it is significant. There are only 0.01% differences in each case. It was found that in-vitro drug release of resveratrol from optimized LCN at pH 7.4 and pH5 was best explained by Higuchi's equation, as the plot showed the highest linearity with a regression coefficient of 0.9528 and 0.9404 respectively. The entrapment efficiency of optimized LCN was found to be  $87.43 \pm 0.52$  % which was in close agreement with the value predicted by design expert software. It was found that the invitro drug release of resveratrol from optimized LCN at pH 7.4 and pH 5 was best explained by Higuchi's equation, as the plot showed the highest linearity with a regression coefficient of 0.9528 and 0.9404 respectively.

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### Introduction:-

Cancer chemotherapy is one of the major way to treat cancer<sup>1</sup>. The aim of the ideal cancer chemotherapy is to deliver the correct amount of drug with desired controlled rate and for sufficiently longer duration of time to the site of action (Tumor) cells, which prevent the normal cells to obtain the desired therapeutic response<sup>2</sup>. Currently around more than 20 different types of nanoparticle therapeutics were clinically in use, validation the ability of nanoparticles to improve the therapeutics index of the drugs<sup>3</sup>. Numerous other nanoparticle already approved nanoparticles including various liposomes<sup>4</sup>. Polymeric micelles, dendrimers, quantum dots, gold nanoparticle and ceramic nanoparticle currently under various preclinical stages of investigation<sup>5</sup>. The first nanoparticles (e.g., Liposomes with encapsulated doxorubicin) had entered the pharmaceutical market in 1995<sup>3</sup>. Since then numerous new nanoparticles for cancer drug delivery have been approved and / or are currently under development due to their many advantages<sup>6</sup>. Their advantages include enhancing solubility of hydrophobic drugs, prolonging circulation time, minimizing non-specific uptake, preventing undesirable off target and side effects, improving intracellular penetration and allowing for specific cancer targeting<sup>6&7</sup>. According to the literatures, there is no drugs were included in this type of formulation but tried for other type such as polymeric formulations with different additives. So, the aim was to Synthesis and Development of a formulation of Nanoparticle containing resveratrol in the liquid crystalline form and finally converted to powder condition for future use

Liquid crystalline nanoparticles (LCNs) are a novel promising drug delivery system. Liquid crystals have structure intermediate between that of a true liquid and solid crystal phase<sup>8</sup>. The liquid crystalline state is a meso-phase between that of an ordered crystal and a disordered isotropic liquid<sup>9</sup>. It has the properties related to that of both liquid and crystals. The liquid state is associated with the ability to flow whereas the solid state characterized by an ordered, crystalline structure<sup>10</sup>. Liquid crystalline nanoparticles have attracted significant attention due to their high drug entrapment efficiency<sup>11</sup>, improved physicochemical stability, tunable and controlled drug release profile and potential for targeting cells and tissues<sup>12</sup>. When cubic and hexagonal liquid crystals are dispersed in to nanoparticles with excess of water with addition of stabilizers such as Fluoronic copolymers and they form stable colloids<sup>13,14,&15</sup>.

### Materials & Methods:-

All chemicals were of analytical grade

**Table 1:-** List of chemicals and manufacturers/suppliers.

List of chemicals	Manufactures/Suppliers
Resveratrol	Procured as a gift sample from Anthem biosciences, Bangalore.
Acetonitrile	Merk specialities (P)Ltd, New Delhi
Diethyl Ether	Central Drug house(P)Ltd, Mumbai
Cellophane Membrane MW CO12-14, kDa	HI Media Laboratories(P) Ltd, Mumbai
Cellophane Membrane MW CO12-14, kDa	HI Media Laboratories(P) Ltd, Mumbai
Ethylenediamine	Merk specialities (P) Ltd, Mumbai
N-hydroxy Succinimide	HI Media Laboratories(P) Ltd, Mumbai
N,N'-dicyclohexyl Carbodimide	Molychem, Mumbai
Trimethylamine	Central drug house(P)Ltd, New Delhi
DMSO	Merkspecialities(P)Ltd, New Delhi
MTT	Sigma-Aldrich, USA
Methanol	Central drug house(P)Ltd, New Delhi
Sodium Hydroxide	Central drug house(P)Ltd, New Delhi
Potassium dihydrogen orthophosphate	Universal chemicals and scientific industries, Haripad
Sodium acetate	Central drug house(P)Ltd, New Delhi

## **Characterisation Of Resveratrol**

### **FTIR spectroscopy**

Sample was mixed with IR grade KBr and made in to a transparent and Homogenous pellet using pressed pellet technique. FTIR scanning was performed by using Agilent technologies CARY630 FTIR, FTIR spectra were taken at  $4\text{ cm}^{-1}$  resolution, averaged over 32 scans in range of  $650\text{ to }4000\text{ cm}^{-1}$ . The spectrum obtained was compared with reported data of Resveratrol.

### **U.V spectrophotometry**

By using JASCO V-630 spectrophotometer, the UV spectrum was prepared with water, PH-5 Phosphate buffer and PH 7.4 Phosphate of 200-400 nm.

### **U.V-Calibration curve of Resveratrol in Methanol**

10 mg of drug was dissolved in methanol and made up to 100ml in a standard flask. 5 ml of this solution was taken and made up to 50 ml in a standard flask to get a concentration of  $10\text{ }\mu\text{g/ml}$  of stock solution. 1,2,3,4 ml of the stock solution was taken and made up to 10 ml in a standard flask to get 1,2,3, and  $4\text{ }\mu\text{g/ml}$  of solutions respectively. The UV absorbance of the solutions were measured at 306 nm using Jasco V-630 spectrophotometer and calibration curve was plotted using the data obtained.

### **U.V –calibration curve in PH 7.4 phosphate buffer**

2. 10 mg of drug was dissolved in PH 5 phosphate buffer and made up to 100ml in a standard flask. 5 ml of the solution was taken and made up to 50 ml in a standard flask. 1,2,3,4  $\mu\text{g/ml}$  was prepared by diluting 1,2,3,4 ml of the above solution to 10 ml. The UV absorbances of the solutions were measured at 311 nm using Jasco V-630 spectrophotometer and calibration curve was plotted using the data obtained.

### **Differential scanning calorimetry**

A Differential Scanning calorimeter, DSC 822e (Mettler Toledo, Switzerland) equipped with Stare software was used to perform the DSC analysis of resveratrol. Nitrogen flow was  $60\text{ ml/min}$  at a heating rate of  $10\text{ }^{\circ}\text{C/min}$ . 5 mg of the sample was accurately weighed, sealed in aluminum pan and equilibrates at  $25\text{ }^{\circ}\text{C}$ , was subjected to DSC run over the temperature range of  $30\text{ to }300\text{ }^{\circ}\text{C}$ . Temperature was calibrated using pure indium with melting point of  $156.60\text{ }^{\circ}\text{C}$ . An empty pan was used as reference.

### **Powder X-ray diffraction**

X ray diffraction data of Resveratrol was performed using Bruker AXS D8 Xray diffractometer. A Cu source with wavelength of  $1.5405\text{ \AA}$  was used. Standard runs were carried out at  $35\text{ mA}$  in this process. Diffractograms were obtained in a scanning range of  $2\theta$  from 3 to 80 at a rate of  $0.02\text{ mn}^{-1}$ .

## **Characterisation Of Monoolein**

### **Solubility**

Solubility of Monoolein was determined quantitatively in various media, like chloroform, ethanol, methanol, water and petroleum ether.

### **FTIR spectroscopy**

Sample was mixed with IR grade KBr and made in to a transparent and Homogenous pellet using pressed pellet technique. FTIR scanning was performed by using Agilent technologies CARY630 FTIR, FTIR spectra were taken at  $4\text{ cm}^{-1}$  resolution, averaged over 32 scans in range of  $650\text{ to }4000\text{ cm}^{-1}$ . The spectrum obtained was compared with reported data of Monoolein.

### **Differential scanning calorimetry:-**

A Differential Scanning calorimeter, DSC 822e (Mettler Toledo, Switzerland) equipped with Stare software was used to perform the DSC analysis of monoolein. Nitrogen flow was  $60\text{ ml/min}$  at a heating rate of  $10\text{ }^{\circ}\text{C/min}$ . 5 mg of the sample was accurately weighed, sealed in aluminum pan and equilibrates at  $25\text{ }^{\circ}\text{C}$ , was subjected to DSC run over the temperature range of  $30\text{ to }300\text{ }^{\circ}\text{C}$ . The temperature was calibrated using pure indium with melting point of  $156.60\text{ }^{\circ}\text{C}$ . An empty pan was used as reference.

### **Characterisation Of Poloxamer 407**

#### **Solubility:-**

Solubility of Poloxamer 407 was determined qualitatively in various media, like chloroform, ethanol, methanol, water and petroleum ether, DMSO.

#### **FTIR spectroscopy:-**

Sample was mixed with IR grade KBr and made in to a transparent and Homogenous pellet using pressed pellet technique. FTIR scanning was performed by using Agilent technologies CARY630 FTIR, FTIR spectra were taken at  $4\text{ cm}^{-1}$  resolution, averaged over 32 scans in range of  $650\text{ to }4000\text{ cm}^{-1}$ . The spectrum obtained was compared with reported data of Poloxamer 407.

#### **Differential scanning Calorimetry:-**

A Differential Scanning calorimeter, DSC 822e (Mettler Toledo, Switzerland) equipped with Stare software was used to perform the DSC analysis of poloxamer 407. Nitrogen flow was  $60\text{ ml/min}$  at a heating rate of  $10\text{ }^{\circ}\text{C/min}$ .  $5\text{ mg}$  of the sample was accurately weighed, sealed in aluminum pan and equilibrates at  $25\text{ }^{\circ}\text{C}$ , was subjected to DSC run over the temperature range of  $30\text{ to }300\text{ }^{\circ}\text{C}$ . The temperature was calibrated using pure indium with melting point of  $156.60\text{ }^{\circ}\text{C}$ . An empty pan was used as reference.

## **Formulation, Design And Development**

### **1.Synthesis And Characterisation Of Folic Acid Conjugated Poloxamer-407**

#### **Synthesis Of CDI –Activated Poloxamer 407:-**

To a solution of poloxamer407(2.01g) in acetonitrile(10ml), a solution of excess of NN'-carbonyl imidazole(CDI,0.26 GM) in acetonitrile(5ml) was added dropwise using a period of 45min.The mixture was kept stirring at room temperature under nitrogen atmosphere for 6 hrs. Then the solution was concentrated in a rotary evaporator and washed with diethyl Ether to remove unreacted CDI, the product was dried in rotary evaporator and collected as a white powder (**Modified Caltagirone et al method**).

#### **Characterisation Of CDI- Activated Poloxamer 407**

##### **FTIR Spectroscopy:-**

The FTIR spectra of pure sample of CDI-activated poloxamer 407 was recorded absorbance bands in the range of  $2886 - 1000\text{ cm}^{-1}$ . The results observed were in close agreement with the reported data.

##### **$^1\text{H}$ NMR spectral analysis:-**

The NMR spectra of the compound shows peak in the range of 1-10 ppm. The results observed were in close agreement with the reported data.

#### **Synthesis Of NH<sub>2</sub>-Terminated Poloxamer 407**

To a solution of CDI activated poloxamer (1.010 gm) in acetonitrile(10ml),0.78 ml of ethylene diamine was added. The reaction mixture was kept stirring for 24 hrs.at room temperature under nitrogen atmosphere. The solvent and unreacted ethylene diamine was removed using rotary evaporator. The product was precipitated with diethyl ether and collected as yellowish powder.

#### **Characterisation Of NH<sub>2</sub> –Terminated Poloxamer 407**

##### **FTIR Spectroscopy:-**

The FTIR spectra of pure sample of NH<sub>2</sub>-terminated poloxamer 407 was recorded absorbance bands in the range of  $2886 - 1000\text{ cm}^{-1}$ . The results observed were in close agreement with the reported data.

##### **$^1\text{H}$ NMR spectral analysis:-**

The NMR spectra of the compound shows peak in the range of 1-10 ppm. The results observed were in close agreement with the reported data.

**Synthesis Of Folate -Conjugated Poloxamer 407:-**

NH<sub>2</sub>-terminated poloxamer (0.498gm), N-hydroxy succinimide (0.33ml), N, N-dicyclo-phenyl carbodiimide (DCC,0.55 gm) and folic acid (FA,0.0533gm) were dissolved in DMSO (10ml) in the presence of triethylamine (50). The reaction mixture was stirred for 24 hrs. at room temperature under nitrogen atmosphere, 20 ml of deionized water was added to the reaction mixture to precipitate dicyclohexylurea and then the mixture was centrifuged and the supernatant was dialyzed for 3 days against de-ionized water which was changed every 3-6 hrs. The resulting product was lyophilized to remove the residual water.

**Characterization Of Folate-Conjugated Poloxamer 407****FTIR Spectroscopy:-**

The FTIR spectra of pure sample of Folate - conjugated poloxamer 407 was recorded absorbance bands in the range of 2886 - 1000 cm<sup>-1</sup>. The results observed were in close agreement with the reported data.

**H-NMR spectral analysis:-**

The NMR spectra of the compound shows peak in thronged of 1-10 ppm. The results observed were in close agreement with the reported data.

**Differential Scanning Calorimetry:-**

The DSC data of Folate conjugated poloxamer 407 was obtained. A sharp endothermic peak appears between 54 - 58°C which corresponds to the melting transition of Folate conjugated poloxamer 407.

**Synthesis Of Liquid Crystalline Nano Particle Dispersion Of Resveratrol**

The required amounts of monoolein (424.662mg) and poloxamer - folic acid conjugated poloxamer (1:1) mixture (210.078 mg) was taken and melted in a water bath at 50 °C followed by resveratrol addition with continuous stirring to complete dissolution. The concentration of resveratrol was fixed at 2 mg/ml from the prototype optimization. Required amount of distilled water was heated to 50°C and gradually added to the melted mixture with vertexing for 10 min. followed by ultra-sonication for 1 hr. The liquid crystalline nanoparticle dispersion prepared was stored at room temperature.

**Optimization Of Folic Acid Cojugated Poloxamer Liquid Crystalline Nano Particle Dispersion Of Resveratrol Using Design Expert Soft Ware 10.0.1.0.Design In Response Surface Method:-**

Central composite design in response surface method was used to optimize the LCN. Amount of monoolein and poloxamer mixture (1:1P407 and P407 FA) were selected as independent factors. Drug entrapment efficiency and drug release were chosen as dependent factors(response). Central composite design suggested 13 formulations based on these factors.

**Characterization Of Optimized Liquid Crystalline Nanoparticle Dispersion****Particle size:-**

The particle size of optimized liquid crystalline nanoparticle was found to be 162.5. The PDI was found to be 0.405, which is less than one, indicating a narrow size distribution and was statistically insignificant.

**Zeta Potential:-**

Zeta potential was determined of the optimized nanoparticle LCN was  $-13.7 \pm 0.06$  mV, resulting in lower aggregation and narrow distribution of particle size. Sufficient zeta potential helps to prevent coalescence of the droplets by electrostatic repulsion.

**Scanning Electron Microscopy:-**

Morphology of optimized LCN was characterized using scanning electron microscope. The samples for SEM image show the presence of clusters of nanoparticles.

**Entrapment Efficiency:-**

Percentage drug entrapment efficiency of prepared formulation (F1, F2 and F3) were established. Experiments were done in triplicate, Average was taken. Percentage drug entrapment efficiency of F red to F2 and F3. Both F2 and F3 have more or less similar entrapment efficiency compared to F1.

**In-Vitro Drug Release:-**

In -vitro drug release was evaluated by dialysis bag of MWCO of 14 kids. The speed was maintained at  $37 \pm 2^\circ\text{C}$  at 100 rpm using magnetic stirrer. 5 ml of liquid crystalline dispersion was placed in the dialysis bag, and receptor compartment was filled with 80 ml of pH 7.4 phosphate buffer with 1% tween 80 to maintain sink condition and the sample were withdrawn at fixed time intervals 0,0.5,1,2,3,4, 5,6,7 hrs. From the receptor to compartment, samples are analyzed UV –spectrophotometrically at 311 nm. Based on the above drug entrapment efficiency and drug release studies. F2 was selected as optimized formulation. F2 had optimum drug entrapment efficiency and drug release with minimum loss of drug. Central composite design in response surface method was used to optimize the LCN. Amount of monoolein and poloxamer mixture (1:1P407 and P407 FA) were selected as independent factors. Drug entrapment efficiency and drug release were chosen as dependent factors(response). Central composite design suggested 13 formulations based on these factors.

**Stability Studies**

The stability studies were carried out at room temperature for a period of 2 months. The nanoparticles were then evaluated for the following parameters.

**Zeta potential:-**

Zeta potential of liquid crystalline nanoparticle dispersion stored at desired temperature and humidity over 60 days were determined. The results show no significant changes in the zeta potential, indicating stability of the product without formation of coalescences. Sufficient zeta potential helps to prevent coalescence of the droplets by electrostatic repulsion.

**Results and Discussion:-****Characterisation Of Resveratrol****FTIR Spectroscopy:-**

The FTIR spectra of pure drug was recorded absorbance bands in the range of  $3184 - 964 \text{ cm}^{-1}$ . The low intensity absorbance bands arising from resveratrol were not much affected by dilution in dry potassium bromide. Therefore, in the present study we have used dry potassium bromide as the diluents. The most prominent absorbance band corresponding to the carbonyl group centered in the range of  $964 - 1610 \text{ cm}^{-1}$ . The results observed were in close agreement with the reported data

**Preparation of calibration plot of resveratrol in methanol**

The following table shows the data of prepared calibration curve of resveratrol in methanol. The UV calibration plot of resveratrol in methanol was found to be linear.

**Table 2:-** Data for calibration plot of resveratrol in methanol.

CONCENTRATION( $\mu\text{g/ml}$ )	ABSORBANCES	R2
1	0.1489	0.9791
2	0.2916	
3	0.4931	
4	0.5785	

**Figure 1:-** U.V. Calibration plot of Resveratrol in methanol.**Table 3:-** Preparation of calibration plot of resveratrol in pH 7.4 phosphate buffer.

COCENTRATION( $\mu\text{g/ml}$ )	ABSRBANCES	R2
1	0.1581	0.9999
2	0.3132	
3	0.4931	
4	0.6348	

The following table shows the data of prepared calibration curve of resveratrol in pH 7.4 phosphate buffer. The UV calibration plot of resveratrol in pH 7.4 phosphate buffer was found to be linear.

#### Differential Scanning Calorimetry

The DSC data of Resveratrol was obtained and a sharp endothermic peak appears between 238-258 °C which corresponds to the melting transition of resveratrol **Powder X-ray Diffraction**

The powder X-ray diffraction pattern of Resveratrol in annexure 8.20 Resveratrol shows numerous diffraction peaks were observed at 20.3, 9.8, 4.73, 5.61, 8.85, 11.18, 12.34, 13.12, 14.56, 15.14, 18.23, 18.74, 20.11, 21.26, 22.01, 23.57, 24.72, 25.87, 26.42 etc. (finger print region). The results observed were in close agreement with reported data.

#### Characterisation Of Monoolein

##### FTIR Spectroscopy

The FTIR spectra of Monoolein was recorded absorbance bands in the range of 3439 - 931  $\text{cm}^{-1}$ . The results observed were in close agreement with the reported data

#### Differential Scanning Calorimetry

DSC analysis of Monoolein was carried out

#### Characterisation Of Poloxamer 407

##### FTIR Spectroscopy

The FTIR spectra of pure sample of poloxamer 407 was recorded absorbance bands in the range of 3569 - 821  $\text{cm}^{-1}$ . The results observed were in close agreement with the reported data

#### Characterisation Of Folic Acid

##### FTIR Spectroscopy

The FTIR spectra of pure sample of folic acid was recorded absorbance bands in the range of 3500 - 900  $\text{cm}^{-1}$ . The results observed were in close agreement with the reported data.

#### Characterisation of CDI-Activated Poloxamer 407

##### FTIR Spectroscopy

The FTIR spectra of pure sample of CDI-activated poloxamer 407 was recorded. The absorbance bands in the range of 2886 - 1000  $\text{cm}^{-1}$ . The results observed were in close agreement with the reported data

#### $^1\text{H}$ NMR spectral analysis

The NMR spectra of the compound shows peak in the range of 1-10 ppm. The results observed were in close agreement with the reported data

**Table 4:-** NMR spectral data of CDI – activated poloxamer 407.

$^1\text{H}$ NMR (DMSO) $\delta$ ppm	NATURE OF PEAK	NATURE OF PROTON PRESENT
1.03	d	Protons of the methyl group of the PPO
3.41 – 3.50	m	-CH <sub>2</sub> -CH(CH <sub>3</sub> )-O- of PPO And -CH <sub>2</sub> -CH <sub>2</sub> -O- of PEO
7.51 -7.69	m	Protons of the imidazole moiety
8.247	s	Protons of the imidazole moiety

#### Synthesis of NH<sub>2</sub>-Terminated Poloxamer 407

NH<sub>2</sub>- terminated poloxamer 407 was synthesized as per the above method.

**FTIR Spectroscopy**

The FTIR spectra of pure sample of NH<sub>2</sub>-terminated poloxamer 407 was recorded absorbance bands in the range of 2886 - 1000 cm<sup>-1</sup>. The results observed were in close agreement with the reported data

**<sup>1</sup>H NMR spectral analysis**

The NMR spectra of the compound shows peak in the range of 1-10 ppm. The results observed were in close agreement with the reported data.

**Table 5:-** NMR spectral data of NH<sub>2</sub> terminated poloxamer 407.

<sup>1</sup> HNMR (DMSO) δppm	NATUTRE OF PEAK	NATURE OF PROTON PRESENT
1.03	d	Protons of the groups of the PPO
2.49 -2.72	m	Protons adjacent to the terminal amine
2.96	q	Protons adjacent to the amide bond
3.41-3.50	m	-CH <sub>2</sub> -(CH)-O- of PPO and - CH <sub>2</sub> -CH <sub>2</sub> -O- of PEO
7.161	t	Amidic -NH

**Synthesis Of Folate -Conjugated Poloxamer 407**

Folate –conjugated poloxamer 407 was synthesized as per above method

**FTIR Spectroscopy**

The FTIR spectra of pure sample of Folate - conjugated poloxamer 407 was recorded absorbance bands in the range of 2886 - 1000 cm<sup>-1</sup>. The results observed were in close agreement with the reported data .

**Table 6:-** FTIR data of folate conjugatedCDI-Activated Poloxamer 407.

WAVE NUMBER cm-1	FUNCTIONAL GROUP
peak at 2886	CH-stretch
Strong peak at 1713	Carbonyl group
3 peak in the range 1000 to 1300	C-O stretching vibration

**<sup>2</sup>H NMR Spectral Anaqlysis**

The NMR spectra of the compound shows peak in the range of 1-10 ppm. The results observed were in close agreement with the reported data.

**In-Vitro Drug Release**

In -vitro drug release was evaluated by dialysis bag of MWCO of 14 kids. The speed was maintained at 37± 2°C at 100 rpm using magnetic stirrer. 5 ml of liquid crystalline dispersion was placed in the dialysis bag, and receptor compartment was filled with 80 ml of pH 7.4 phosphate buffer with 1% tween 80 to maintain sink condition and the sample were withdrawn at fixed time intervals 0,0.5,1,2,3,4,5,,6,7 hrs. From the receptor to compartment. Samples are analyzed UV –spectrophotometrically at 311 nm.

**LIQUID CRYSTALLINE NANOPARTICLE FORMULATIONS.**

For the study purpose, three Formulations F1,F2,F3 were prepared as per above method and best is selected for original formulation as follows

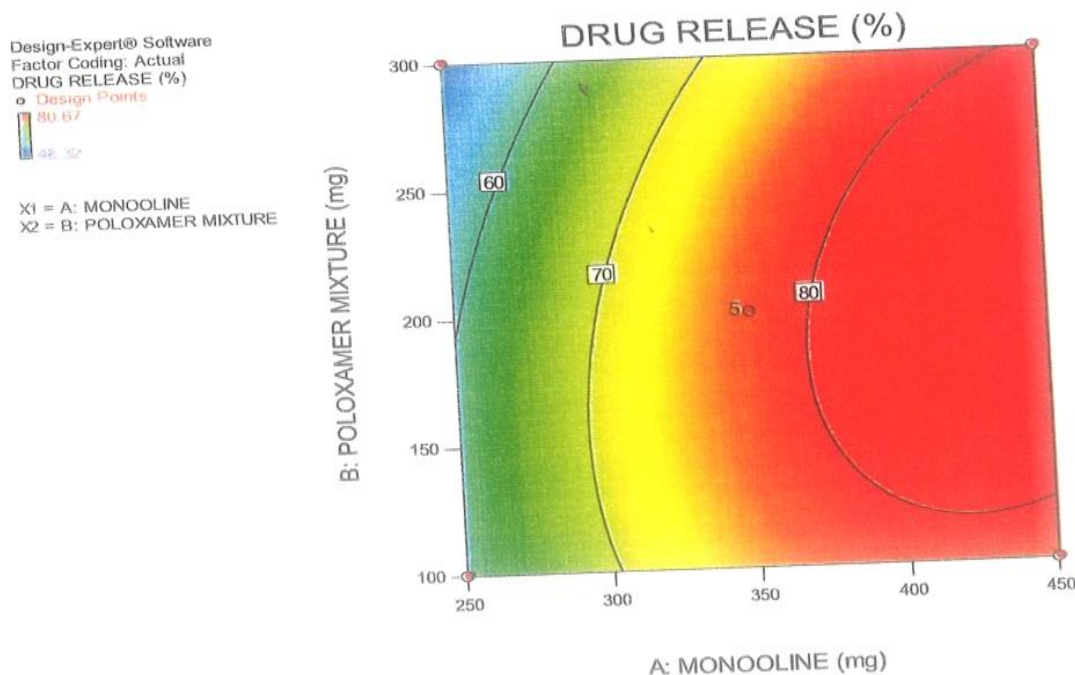
**Table.7. Final formula of optimized liquid crystalline nanoparticle dispersion**

MONOOLEIN 424.062 mg	POLOXAMER :FOLIC ACID CONJUGATED POLOXAMER (1:1) 210.078	WATER 9.3 ml
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The required amounts of monoolein (424.662mg) and poloxamer - folic acid conjugated poloxamer (1:1) mixture(210.078 mg) was taken and melted in a water bath at 50 °C followed by resveratrol addition with continuous stirring to complete dissolution. The concentration of resveratrol was fixed at 2 mg/ml from the prototype optimization. Required amount of distilled water was heated to 50°C and gradually added to the melted mixture with vortexing for 10 min. followed by ultra-sonication for 1 hr.

**Figure 3:-** Percentage Drug release.

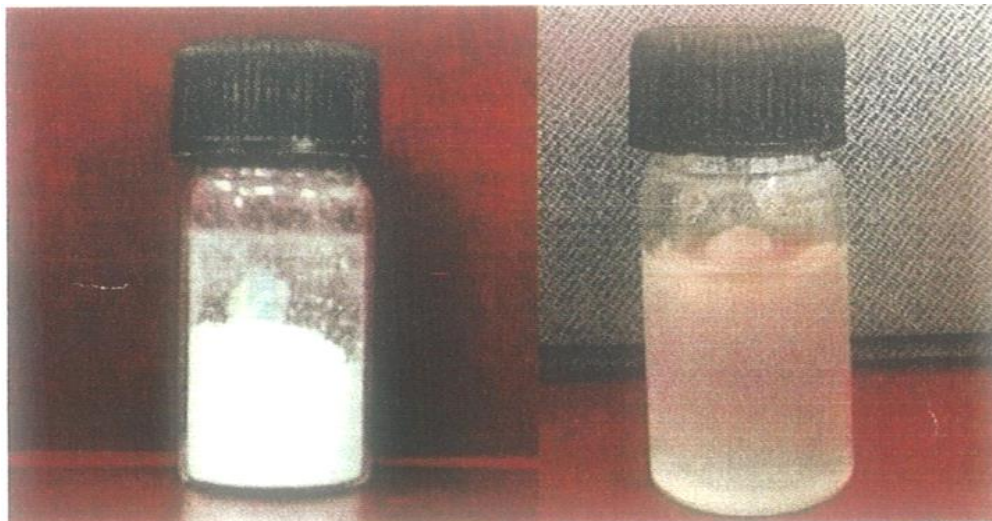


**ENTRAPMENT EFFICIENCY** =  $77.922 + 11.8675A - 1.9348B + 3.155 AB - 4.24787 A^2 - 4.24787 B^2$       Where  
 A= MONOOLEIN B= POLOXAMER MIXTURE

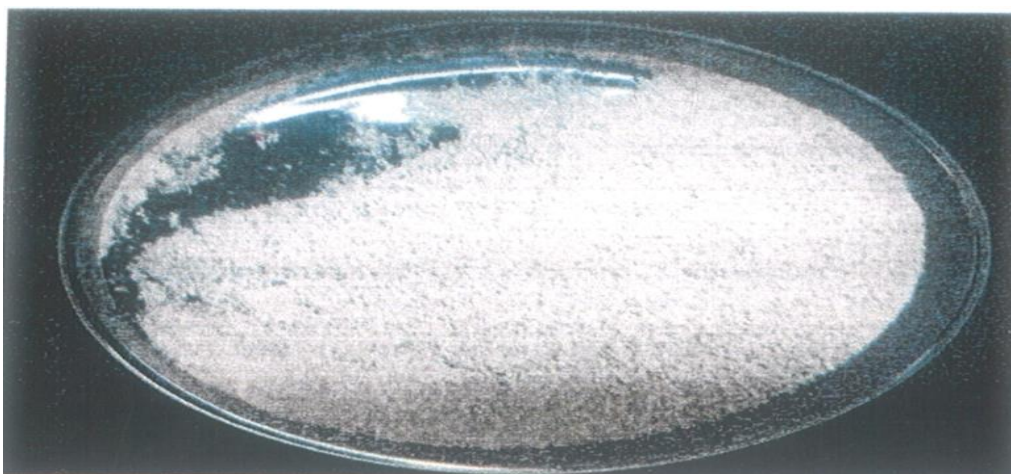
**Conversion Of Lcn Dispersion In To Granules**

**Table 8:-** Formula for LCN granules.

AMOUNT OF LCN DISPERSION	AMOUNT OF STARCH
5 ML	7.89 GM



**Figure 7:-** Optimized Liquid crystalline nanoparticle dispersion.



**Figure 8:-** Dried granules of LCN with starch.

### **Conclusion:-**

The resveratrol loaded folic acid conjugated liquid crystalline nanoparticles were prepared by ultra-sonication method. Initially the characterization of resveratrol, monoolein and poloxamer 407 were done using UV-Visible spectrophotometry, FTIR spectroscopy, DSC analysis. Folic acid was characterized by U.V-Visible spectrophotometry, solubility determinations and FTIR spectroscopy. The result was close agreement with the reported data. Folic acid conjugated poloxamer 407 was prepared by carbamate chemistry in a three-step reaction scheme. The folate textured poloxamer 407 was characterized using FTIR spectroscopy, DSC analysis and  $^1\text{H}$  NMR spectroscopy. The optimization parameters like drug entrapment efficiency and in vitro drug release were evaluated. The software provided 100 solutions for optimized formulation, out of which the one with maximum desirability of one was selected. The model predicted 87.61% of drug entrapment efficiency and drug release of 83.17%. Optimized formula provided by the software was to prepare LCN by ultra-sonication method. Optimum amount of 424.062 mg of monoolein and 210.073 mg of poloxamer mixture were selected.

The characterization of optimized LCN was performed. As it had a mean particle size of 125.5 nm, it can undergo Enhanced permeation Retention (EPR) effect in solid tumors. The PDI (polydispersity index) of liquid of liquid crystalline formulation was 0.405, which is less than one, indicating that a narrow size distribution. Zeta potential was found to be  $-13.7 \pm 0.06$  mV. The optimized LCN showed an entrapment efficiency of  $87.43 \pm 0.52\%$ . In vitro cumulative percentage drug release of  $83.45 \pm 0.43\%$  in pH 7.4 phosphate buffer and  $85.18 \pm 0.50\%$  in pH 5 acetate

buffer were obtained at the end of 7 hrs. The scanning electron microscopy and transmission electron microscopy confirmed the cuboidal morphology of nanoparticle

The stability studies were carried out on the optimized liquid crystalline nanoparticle for 2 months in room temperature. The LCN dispersion was converted to solid granules using starch. The granules are evaluated for its flow properties. The angle of repose of LCN granules was found to be 29.28° which indicate good flow properties. It had a bulk density of 0.472 g/ml and tapped density of 0.491 g/ml. The Car's compressibility index (%) of LCN granules was found to be 4.91% and a Hauser's ratio of which indicates that it had good flow.

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