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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. In this experiment, all the materials used in the experiments were of analytical grade. Synthesis of CDI-activated poloxamer-407, NH2 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 ± 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.

INTRODUCTION

Cancer chemotherapy is one of the major way to treat cancer[1]. 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[2]. 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[3]. Numerous other nanaoparticles, already approved includes various liposomes, polymeric micelles, dentrmers, quantum dots, gold nanoparticles and ceramic nanoparticles are currently under various preclinical stages of investigations. The first

nanoparticles (e.g., Liposomes with encapsulated doxorubicin) had entered the pharmaceutical market in 1995[3]. Sincethen numerous new nanoparticles for cancer drug delivery have been approved and / or are currently under development due to their many advantages[6]. 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[6&7]. 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) area novel promising drug delivery system. Liquid crystals have structure intermediate between that of a true liquid and solid crystal phase[8]. The liquid crystalline state is a mesa-phase between that of an ordered crystal and a disordered isotropic liquid[9]. 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[10]. Liquid crystalline nanoparticles have attracted significant attention due to their high drug entrapment efficiency[11], improved physicochemical stability, tunable and controlled drug release profile and potential for targeting cells and tissues[12] .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[13,14,&15].

MATERIALS & METHODS

All chemicals were of analytical grade.

1. CHARACTERISATION OF RESVERATROL

1.1. FTIR spectroscopy

Sample was mixed with IR grade KBr and made in to a

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, NewDelhi
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

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

1.2. 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.

1.3. 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 µg/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 4µg/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.

1.4. 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 µg/ 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.

1.5. 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 ml/min at a heating rate of 10 ^{0}C/min.5 mg of the sample was accurately weighed, sealed in aluminum pan and equilibrates at 25 ^{0}C, was subjected to DSC run over the temperature range of 30 to 300 ^{0} C. Temperature was calibrated using pure indium with melting point of 156.60 ^{0} C. An empty pan was used as reference.

1.6. Powder X-ray diffraction

X ray diffraction data of Resveratrol was performed using Bruker AXS D8 X ray diffractometer. A Cu source with wavelength of 1.5405 A^{0}was used. Standard runs were carried out at 35 mA in this process. Diffractograms were obtained in a scanning range of 2Θ from 3 to 80 at a rate of 0.02 mn^{-1}.

2. CHARACTERISATION OF MONOOLEIN

2.1. SOLUBILITY

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

2.2. 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 cm^{-1} resolution, averaged over 32 scans in range of 650 to 4000 cm^{-1}. The spectrum obtained was compared with reported data of Monoolein.

2.3. 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 ml/min at a heating rate of 10 ^{0}C/min.5 mg of the sample was accurately weighed, sealed in aluminum pan and equilibrates at 25 ^{0}C, was subjected to DSC run over the temperature range of 30 to 300 ^{0} C. The temperature was calibrated using pure indium with melting point of 156.60 ^{0} C. An empty pan was used as reference.

3. CHARACTERISATION OF POLOXAMER 407

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

3.2. 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 cm^{-1} resolution, averaged over 32 scans in range of 650 to 4000 cm^{-1}. The spectrum obtained was compared with reported data of Poloxamer 407.

3.3. 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 ml/min at a heating rate of 10 ^{0}C/min.5 mg of the sample was accurately weighed, sealed in aluminum pan and equilibrates at 25 ^{0}C, was subjected to DSC run over the temperature range of 30 to 300 ^{0} C. The temperature was calibrated using pure indium with melting point of 156.60 ^{0} C. An empty pan was used as reference.

4. FORMULATION, DESIGN AND DEVELOPMENT

SYNTHESIS AND CHARACTERISATION OF FOLIC ACID CONJUGATED POLOXAMER-407

4.1. 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).

4.2. CHARACTERISATON OF CDI-ACTVATED POLOXAMER 407

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

4.2.2. ^{1}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.

4.3. SYNTHESIS OF NH2-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.

4.4. CHARACTERISATION OF NH2 TERMINATED POLOXAMER 407

4.4.1FTIR Spectroscopy: -The FTIR spectra of pure sample of NH2-terminated poloxamer 407 was recorded absorbance bands in the range of 2886 - 1000 cm^{-1}. The results observed were in close agreement with the reported data.

4.4.2. ^{1}H NMR spectral analysis: -The NMR spectra of the compound shows peak in therange of 1-10 ppm. The results observed were in close agreement with the reported data.

4.5 SYNTHESIS OF FOLATE -CONJUGATED POLOXAMER 407: - NH2-terminated poloxamer (0.498gm), N-hydroxy succinimide (0.33ml), N, N-dicycle-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.

4.5. CHARACTERISATION OF FOLATE-CONJUGATED POLOXAMER 407

4.5.1. FTIR Spectroscopy: -The FTIR spectra of pure sample of Folate - conjugated poloxamer 407 was recorded absorbance bands in the range of 2886 - 1000 cm^{-1}. The results observed were in close agreement with the reported data.

4.5.2 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.

4.5.3. Differential Scanning Calorimetry: -The DSC data of Folate conjugated poloxamer 407 was obtained. A sharp endothermic peak appears between 54 - 58ºCwhich corresponds to the melting transition of Folate conjugated poloxamer 407.

4.6. 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 ºC 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 50ºC 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.

4.7. 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.

4.8. CHARACTERIZATION OF OPTIMIZED LIQUID CRYSTALLINE NANOPARTICLE DISPERSION

4.8.1Particle 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.

4.8.2. Zeta Potential: - Zeta potential was determined of the optimized nanoparticle LCN was -13.7 ± 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.

4.8.3. 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.

5. 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.

6. IN-VITRO DRUG RELEASE: -

In -vitro drug release was evaluated by dialysis bag of MWCO of 14 kids. The speed was maintained at 37± 2ºC 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.

7. 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

CHARACTERISATION OF RESVERATROL

1. FTIR Spectroscopy :- The FTIR spectra of pure drug was recorded absorbance bands in the range of 3184 - 964 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 cm-1. The results observed were in close agreement with the reported data

2. 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. (Fig. 2)

Table 2 : Data for calibration plot of resveratrol in methanol

CONCENTRATION(µg/ml)	ABSORBANCES	R2
1	0.1489	0.9791
2	0.2916	
3	0.4931	
4	0.5785	

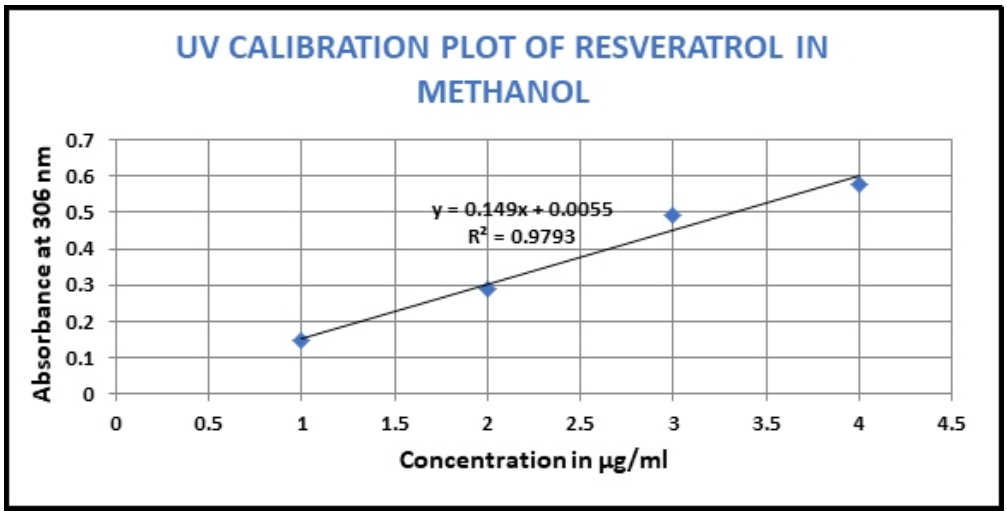

Figure 1 : U.V. Calibration plot of Resveratrol in methanol

Table 3 : Data for calibration plot of resveratrol in methanol

COCENTRATION(µg/ml)	ABSRBANCES	R2
1	0.1581	0.9999
2	0.3132	
3	0.4931	
4	0.6348	

Differential Scanning Calorimetry

The DSC data of Resveratrol was obtained and a sharp endothermic peak appears between 238-258 ºC 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, 3.98, 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 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 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 cm^{-1}.

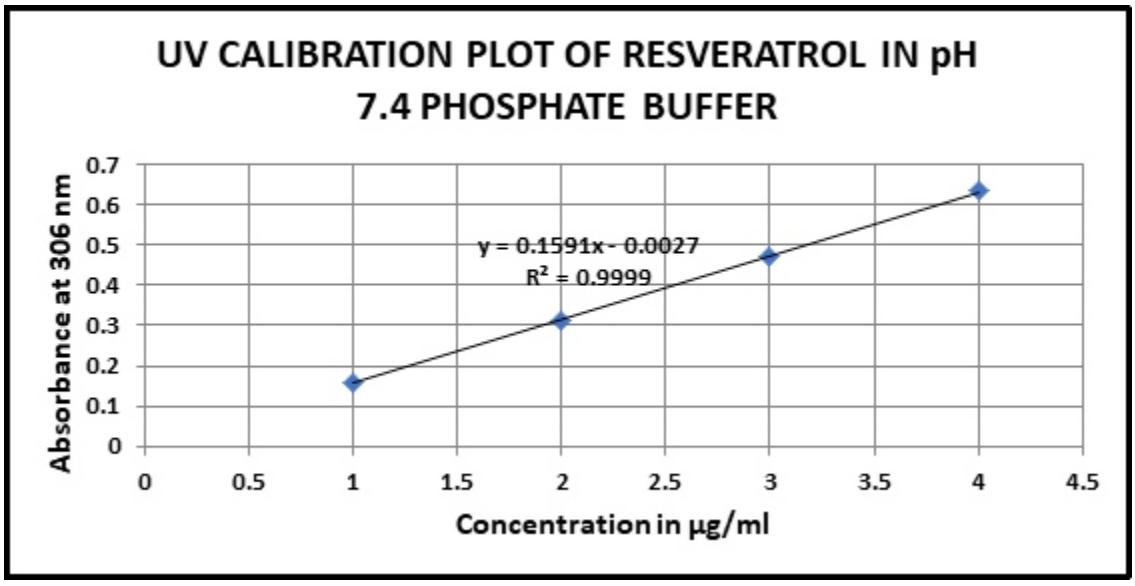

Figure 2 : U.V. Calibration plot of Resveratrol in pH 7.4 Phosphate buffer

Table 4 : CNMR spectral data of CDI activated poloxamer 407

1HNMR (DMSO) ð ppm	NATURE OF PEAK	NATURE OF PROTON PRESENT
1.03	d	Protons of the methyl group of the PPO
3.41 – 3.50	m	$-CH_2-CH(CH_3)-O-$ of PPO And $–CH_2 –CH_2 –O-$ of PEO
7.51 -7.69	m	Protons of the imidazole moiety
8.247	s	Protons of the imidazole moiety

The results observed were in close agreement with the reported data.

CHARACTERISATONOF CDI-ACTVATED PO OXAMER 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 cm^{-1}. The results observed were in close agreement with the reported data

^{1}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 .

SYNTHESISOF NH2-TERMINATED POLOXAMER 407

NH_2 terminated poloxamer 407 was synthesized as per the above method.

FTIR Spectroscopy

The FTIR spectra of pure sample of NH2-terminated poloxamer 407 was recorded absorbance bands in the range of 2886 - 1000 cm^{-1}. The results observed were in close agreement with the reported data

^{1}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.

4. 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^{-1}. The results observed were in close agreement with the reported data.

Table 5 : NMR spectral data of NH2 terminated poloxamer 407

^{1}HNMR (DMSO) ðppm	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	-CH2-(CH)-O- of PPO and - CH2-CH2-O- of PEO
7.161	t	Amidic –NH

Table 6 : FTIR data of folate conjugated CDI - 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

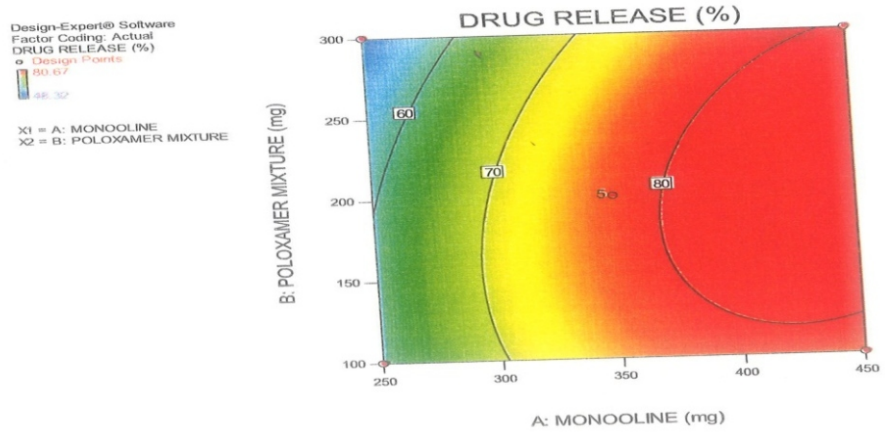

Figure 3 : Percentage Drug release

Table 7 : Final formula of optimized liquid crystalline nanoparticle dispersion

MONOOLEIN	POLOXAMER :FOLIC ACID CONJUGATED POLOXAMER (1:1)	WATER
424.062 mg	210.078	9.3 ml

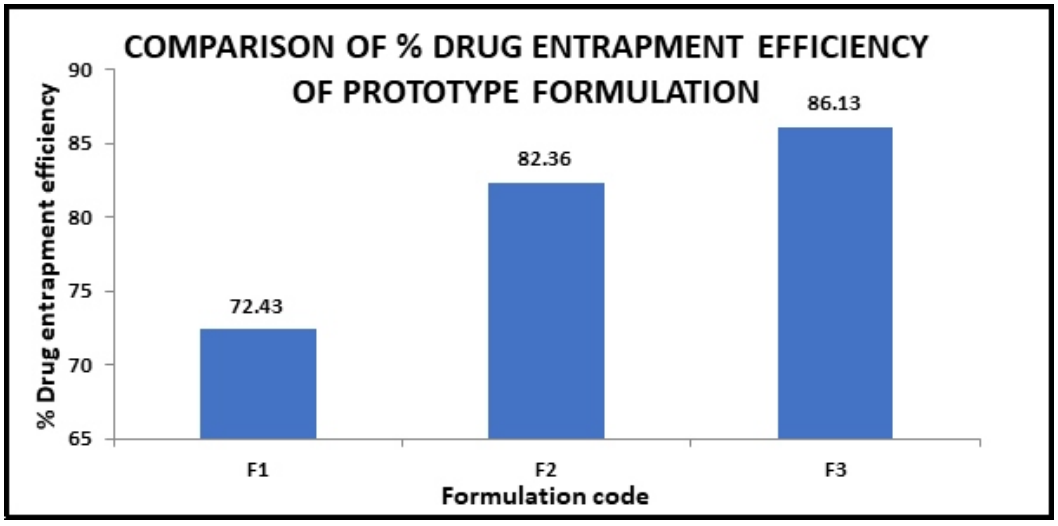

Figure 4 : Comparison of %Drug entrapment Efficiency of Prototype Formulation

2[1]H NMR SPECTRALANAQLYSIS

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.

5. IN-VITRO DRUG RELEASE

In -vitro drug release was evaluated by dialysis bagof MWCO of 14 kids. The speed was maintained at 37± 2ºC 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.

6. 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.

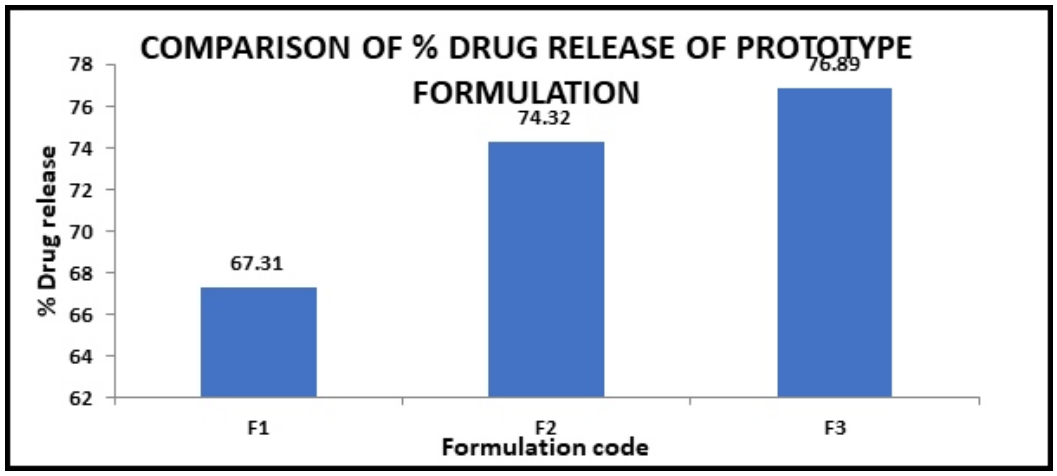

Figure 5 : Comparison of % Drug Release of Prototype Formulation

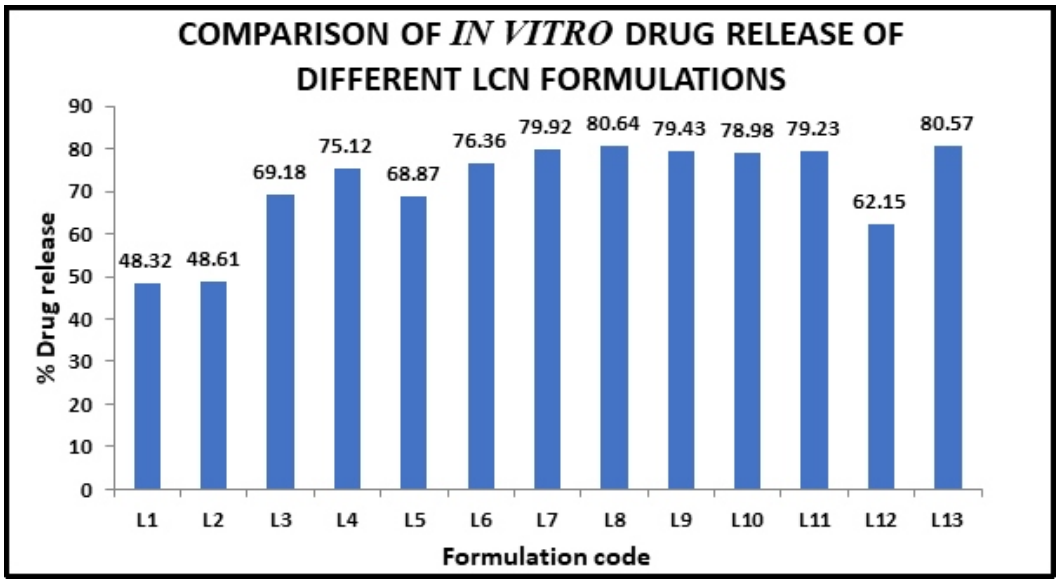

Figure 6 : Comparison of Drug release of different formulations

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 ºC 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 50ºC and gradually added to the melted mixture with vortexing for 10 min. followed by ultra-sonication for 1 hr.

DRUG 7. 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.

8. 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

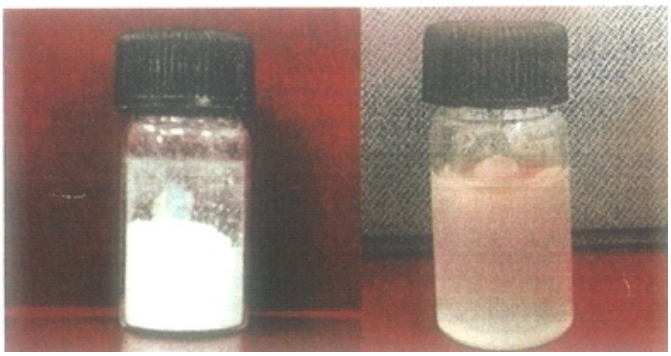

Figure 7 : Optimized Liquid crystalline nanoparticle dispersion

Figure 8 : Dried granules of LCN with starch

DISCUSSIONS

Resveratrol (RES) - ($C_{14}H12O_3$) is 3,4',5-trihydroxystilbene, molecular weight 228.24 g/mol, IUPAC name,3,5,4'-stilbe-netriol;5-[(1E)-2-(4-hydroxyphenyl)ethenyl]-1,3benzenediol; (E)-5-(p-Hydroxy styryl) resorcinol, melting point, 253 - 255º C, practically insoluble in water (3 mg/100ml), but soluble in Ethanol (50 mg/ml), DMSO (16 mg/ml).Resveratrol is a naturally occurring polyphenol, which has been found in some fruits, vegetables and traditional oriental medicinal plants such as grapes, nuts and polygonium roots. The anti-tumor activity of RES has been noticed. Though the antitumor mechanism was investigated, it was explained complexly and diversely. In brief, the inhibition effect on the enzymatic activity of cyclooxygenase was considered as a major reason for RES to reduce the probability of cancer growth. The promising anti-tumor effect of RES has aroused much interest in recent years.

Unfortunately, most studies were restricted on in-vitro cellular level due to the poor water solubility of RES. RES can be well dissolved in various common organic solvents such as ether, ethanol, methanol, dimethyl sulfoxide (DMSO), but barely dissolved in water. In order to achieve effective pharmacological doses of RES, a considerable amount of organic solvent is usually required in cell line tests which is not suitable and acceptable for in vivo drug delivery. Therefore, the clinical application of RES has been seriously hindered. The oral absorption of resveratrol in humans is about 75% and is thought to occur mainly by trans-epithelial diffusion. Active transport might occur as well, but likely only by resveratrol metabolites. The oral bioavailability of resveratrol is almost zero due to rapid and extensive metabolism and the consequent formation of various metabolites as resveratrol glucuronides and resveratrol sulfates. So, my study includes all the consideration of these factors and according to literature I found that there is not any single formulation under this category.

The 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. The liquid crystalline state is a mesa-phase between that of an ordered crystal and a disordered isotropic liquid. It has the properties related to taint-tumor activity of RES has been noticed. Though the antitumor mechanism was investigated, it was explained complexly and diversely 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. Liquid crystalline nanoparticles have attracted significant attention due to their high drug entrapment efficiency, improved physicochemical stability, tunable and controlled drug release profile and potential for targeting cells and tissues .When cubic and hexagonal liquid crystals are dispersed in to nanoparticles with excess of water with addition of stabilizers such as Fluorotic copolymers and they form stable colloids.

Nano-encapsulation of anti-proliferative and chemo-preventive phytoalexin trans resveratrol is likely to provide protection against degradation, enhancement of bioavailability improvement in intracellular penetration and controlled delivery. 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, melting point and solubility parameters determinations. 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 carbidomide chemistry in a three-step reaction scheme. The folate textured poloxamer 407 was characterized using FTIR spectroscopy, DSC analysis and ^{1}H- NMR spectroscopy. Prior to formulation the chemical compatibility of resveratrol with excipients were performed using FTIR spectroscopy and DSC analysis, which showed no significant interactions.

Present study demonstrates that textured poloxamer based LCN had enhanced anti-proliferative effect than the LCN without a targeting ligand and that of resveratrol solution. Solubility, stability and intracellular delivery of resveratrol were increased by its loading in to LCN formulation. The folate textured poloxamer LCN can provide an improved targeted release of resveratrol in tumor cells by use of targeting ligand, EPR effect and by controlled drug release with good bio-compatibility. The encouraging results obtained from the study of LCN granules indicated that it could be proposed as capsule formulation. The folate textured poloxamer based LCN offers a promising potential as tumor targeted drug delivery platform for resveratrol as a result of its multiple synergistic factors. So, this formulation can be utilized for the future drug development and synthesis of formulations.

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 carbetamide chemistry in a three-step reaction scheme. The folate textured poloxamer 407 was characterized using FTIR spectroscopy, DSC analysis and ^{1}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±0.06 mV. The optimized LCN showed an entrapment efficiency of 87.43 ±0.52%. *In vitro* cumulative percentage drug release of 83.45±0.43% in pH 7.4 phosphate buffer and 85.18±0.50% in pH5 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 29.28º 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 f64nd t6 be 4.91% and a Hauser's ratio of which indicates that it had good flow.

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